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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Systemic and Terminal Ileum Mucosal Immunity Elicited by Oral Immunization With the Ty21a Typhoid Vaccine in Humans

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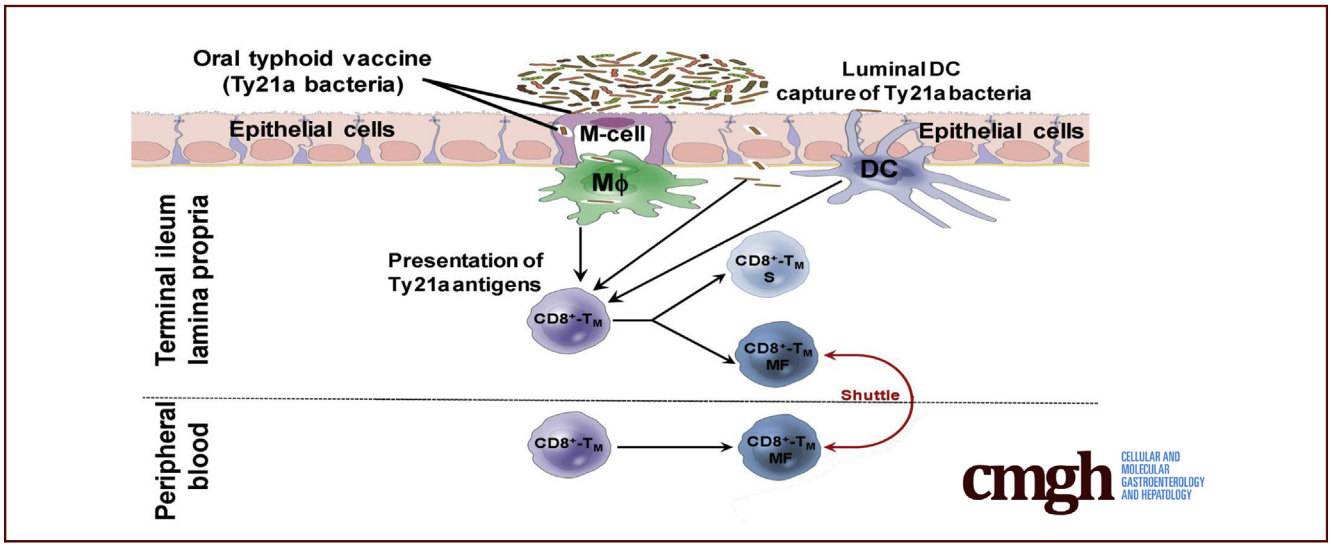

SUMMARY

This study examines mucosal immune responses to administration of the oral Ty21a-typhoid vaccine in humans. Local antigen-specific $CD8^+$-T_M responses were substantially different from those observed systemically. These data have broad implications in human mucosal immune regulation and approaches to oral immunization.

BACKGROUND & AIMS: Systemic cellular immunity elicited by the Ty21a oral typhoid vaccine has been extensively characterized. However, very limited data are available in humans regarding mucosal immunity at the site of infection (terminal ileum [TI]). Here we investigated the host immunity elicited by Ty21a immunization on terminal ileum–lamina propria mononuclear cells (LPMC) and peripheral blood in volunteers undergoing routine colonoscopy.

METHODS: We characterized LPMC-T memory (T_M) subsets and assessed *Salmonella enterica* serovar Typhi (*S* Typhi)–specific responses by multichromatic flow cytometry.

RESULTS: No differences were observed in cell yields and phenotypes in LPMC $CD8^+$-T_M subsets following Ty21a immunization. However, Ty21a immunization elicited LPMC $CD8^+$ T cells exhibiting significant *S* Typhi–specific responses (interferon-γ, tumor necrosis factor-α, interleukin-17A, and/or CD107a) in all major T_M subsets (T-effector/memory [T_{EM}], T-central/memory, and T_{EM}-$CD45RA^+$), although each T_M subset exhibited unique characteristics. We also investigated whether Ty21a immunization elicited *S* Typhi–specific multifunctional effectors in LPMC $CD8^+$ T_{EM}. We observed that LPMC $CD8^+$ T_{EM} responses were mostly multifunctional, except for those cells exhibiting the characteristics associated with cytotoxic responses. Finally, we compared mucosal with systemic responses and made the important observation that LPMC $CD8^+$ *S* Typhi–specific responses were unique and distinct from their systemic counterparts.

CONCLUSIONS: This study provides the first demonstration of *S* Typhi–specific responses in the human terminal ileum mucosa and provides novel insights into the generation of mucosal immune responses following oral Ty21a immunization. *(Cell Mol Gastroenterol Hepatol 2017;4:419–437; http://dx.doi.org/10.1016/j.jcmgh.2017.08.002)*

Keywords: Lamina Propria Mononuclear Cells; Multifunctional T Cells; $CD8^+$-T Memory Cells; Typhoid; Vaccines.

See editorial on page 439.

The causative agent of typhoid fever, *Salmonella enterica* serovar Typhi (*S* Typhi), is a human restricted pathogen that constitutes a major global health threat. Annually, *S* Typhi infection leads to an estimated 26.9 million cases of typhoid fever resulting in approximately 217,000 deaths worldwide.[1,2] Following ingestion, *S* Typhi invades the host mucosal surfaces mostly via M cells, which are specialized epithelial cells covering the Peyer patches. Subsequently, *S* Typhi translocates to the submucosa where it encounters intestinal lymphoid tissues, before entering draining mesenteric lymph nodes, and disseminating to the liver, spleen, and other secondary lymphoid tissues, resulting in systemic illness.[3] Although *S* Typhi can potentially invade at any site harboring M cells along the intestine,[4] the human terminal ileum (TI), where most of Peyer patches in the intestine are concentrated,[5] is the favored intestinal active invasion site for *S* Typhi.[3] In *S* Typhi–infected patients in developing countries, one of the most common complications of typhoid fever are multiple intestinal perforations that occur almost exclusively in the TI. This evidence from the clinic argues strongly that the TI is the major site of infection for *S* Typhi. Only very limited information is available regarding the generation of cell-mediated immune responses (CMI) to *S* Typhi in the human intestinal mucosa.[6,7] Moreover, to our knowledge, there are no data on the induction of CMI responses to *S Typhi* in the TI mucosa following wt *S* Typhi infection or immunization with the live attenuated oral vaccine Ty21a (Ty21a). Thus, a better understanding of the host mucosal immune responses against *S* Typhi and other enteric pathogens at their preferred site of natural infection is required to provide additional insights for the development of oral vaccines.

Currently, 2 licensed typhoid vaccines are available in the United States for use in humans including Ty21a.[8,9] Ty21a is typically administered in 4 spaced doses and confers a moderate level of long-lived protection (60%–80%; 5–7 years).[10–12] Hence, there is a need to develop effective new vaccines that provide durable, long-lasting protection. The assessment of mucosal immune responses at the site of infection (TI) may allow the identification of immune correlates of protection, which has the potential to greatly contribute to the development of new generations of attenuated typhoid vaccines. Our group and others have extensively studied the induction of humoral and CMI responses in peripheral blood mononuclear cells (PBMC) obtained from healthy volunteers following immunization with 4 doses of Ty21a.[13–19] These studies showed that live oral *S* Typhi vaccines induced both $CD4^+$ and $CD8^+$ T-cell responses, including cytotoxic T cells, proliferation, and multifunctional (MF) antigen-specific cytokine-producing cells.[12–14,16,20–22] We also reported that Ty21a elicits *S* Typhi–specific $CD8^+$ T-cell responses in PBMC by various $CD8^+$ T memory (T_M) cell subsets, including T central memory (T_{CM}), T effector/memory (T_{EM}), and RA^+T_{EM} (T_{EMRA})[16,23] and that these responses are predominantly in the T_{EM} and T_{EMRA} subsets with a low magnitude of responses observed in $CD8^+$ T_{CM} subsets.[12,21,23] Recent reports have indicated that various vaccines, including Ty21a, have the capacity to induce antigen-specific MF T cells (cells that produced 2 or more responses), which might play a key role in long-term immunity.[12,21,23] However, these detailed CMI responses were assessed in peripheral blood. CMI responses in the human TI have never been directly investigated. We hypothesized that *S* Typhi–specific responses by various $CD8^+$ T_M subsets elicited in the TI following Ty21a immunization would differ in magnitude and characteristics from their systemic counterparts.

In this study we have characterized TI–lamina propria mononuclear cells (LPMC) T_M in Ty21a-vaccinated and unvaccinated volunteers. We then determined and compared $CD8^+$ T_M *S* Typhi–specific responses from the 2 groups following stimulation with autologous target cells infected with wild-type (wt) *S* Typhi. Finally, we assessed these responses in depth by analyzing their multifunctionality and directly compared peripheral and mucosal $CD8^+$ T_{EM} MF responses. These comparisons provide a unique insight between mucosal and peripheral immunity.

Materials and Methods

Volunteers, Immunization, and Sample Collection

Healthy volunteers undergoing routine, medically indicated colonoscopies who had no history of typhoid fever were recruited from the Baltimore-Washington metropolitan area and University of Maryland, Baltimore campus. Written informed consent was obtained from volunteers and all procedures were approved by the University of Maryland, Baltimore Institutional Review Board. Volunteers (aged 49–74 years) were assigned into 2 groups. The first group (n = 13) received the 4 recommended doses of Ty21a vaccine (Vivotif enteric-coated capsules, Crucell, Bern, Switzerland), whereas volunteers assigned to the second group were not vaccinated (control group) (n = 22) as shown in the study design (Figure 1*A*). Blood samples were collected at least 21 days before immunization (preimmunization) and on colonoscopy day (Day 0; postvaccination). Using large capacity forceps, TI biopsies were obtained only on colonoscopy day (Day 0; postvaccination) as stated in the approved Institutional Review Board protocol (Figure 1*A*). PBMC were isolated immediately after blood draws by density gradient centrifugation and cryopreserved in liquid nitrogen following standard techniques.[21]

Isolation of LPMC From Terminal Ileum Biopsies

TI-LPMC were freshly isolated as described previously.[24,25] Briefly, after collection of biopsies from routine

Abbreviations used in this paper: **CMI, cell-mediated immune responses; EBV-B, Epstein-Barr virus–transformed lymphoblastoid B cells; IFN, interferon; IL, interleukin; LPMC, lamina propria mononuclear cells; MF, multifunctional; MIP, macrophage inflammatory protein; PBMC, peripheral blood mononuclear cells; S, *S* Typhi–specific single producing cells; T_{CM}, T-central/memory ($CD62L^+CD45RA^-$); T_{EM}, T-effector/memory ($CD62L^-CD45RA^-$); T_{EMRA}, T_{EM}-$CD45RA^+$ ($CD62L^-CD45RA^+$); T_M, $CD8^+$ T memory; TI, terminal ileum; TNF, tumor necrosis factor; wt, wild-type.**

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