Clinical Immunology (2013) **148**, 35–43

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Shigella antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type *Shigella flexneri* 2a

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Received 24 September 2012; accepted with revision 12 March 2013
Available online 26 March 2013

KEYWORDS
Shigella;
Protection;
Human challenge;
B memory;
LPS;
IpaB

Abstract The role of *Shigella*-specific B memory (B_M) in protection has not been evaluated in human challenge studies. We utilized cryopreserved pre- and post-challenge peripheral blood mononuclear cells and sera from wild-type *Shigella flexneri* 2a (wt-2457T) challenges. Challenged volunteers were either naïve or subjects who had previously ingested wt-2457T or been immunized with hybrid *Escherichia coli–Shigella* live oral candidate vaccine (EcSf2a-2). B_M and antibody titers were measured against lipopolysaccharide (LPS) and recombinant invasion plasmid antigen B (IpaB); results were correlated with disease severity following challenge. Pre-challenge IgA IpaB-B_M and post-challenge IgA LPS-B_M in the previously exposed subjects negatively correlated with disease severity upon challenge. Similar results were observed with pre-challenge IgG anti-LPS and anti-IpaB titers in vaccinated volunteers. Inverse correlations between magnitude of pre-challenge IgG antibodies to LPS and IpaB, as well as IgA IpaB-B_M and post-challenge IgA LPS-B_M with disease severity suggest a role for antigen-specific B_M in protection.

Abbreviations: ASC, antibody secreting cell; B_M, memory B cells; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunosorbent spot assay; Ig, immunoglobulin; Ipa, Invasion plasmid antigen; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; SFC, spot forming cells

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1521-6616/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clim.2013.03.009

1. Introduction

Shigella is an important cause of morbidity and mortality from diarrheal diseases among children living in developing countries [1,2]. The control of shigellosis is impeded by the emergence of antibiotic resistance [3] and lack of a commercially available vaccine. Obstacles in *Shigella* vaccine development include the lack of adequate animal challenge models that faithfully reproduce human shigellosis and the complexity of performing human challenge studies or prospective clinical studies in the field [4]. Challenge studies offer a means of studying protective immunity in humans. Three challenge studies were performed at the University of Maryland School of Medicine Center for Vaccine Development (CVD) in the early 1990s to evaluate the efficacy of a live oral hybrid *Escherichia coli–Shigella flexneri* 2a vaccine candidate (EcSf2a-2) developed at the Walter Reed Army Institute of Research [5] and to refine the wild-type challenge model. Efficacy was assessed by measuring the ability of either the vaccine or wild-type infection to prevent illness following experimental challenge with wild-type *S. flexneri* 2a strain 2457T (wt-2457T) [6,7]. In two studies, volunteers were administered multiple spaced doses of EcSf2a-2 and challenged one month later (along with a group of unvaccinated controls) with wt-2457T. The vaccine induced a modest immune response and conferred 27–36% efficacy against challenge [7]. A subset of these volunteers who developed gastrointestinal symptoms of shigellosis (diarrhea or dysentery) after challenge with virulent *S. flexneri* 2a in bicarbonate buffer agreed to participate in a second challenge study with wt-2457T along with a group of subjects who had not been previously immunized or challenged; the protective efficacy of prior exposure to wt-2457T reached 70% [6]. Based on these studies, IgA anti-LPS antibody secreting cell (ASC) responses have been proposed as a possible correlate of protection [7]. Heretofore, very limited additional putative immune markers, notably IgG serum antibody titers against lipopolysaccharide (LPS) have been found in most, but not all, studies to be statistically correlated with protection against *Shigella* infection [4,8–11]. Other immune mechanisms that have been proposed to correlate with protection against shigellosis include serum antibody responses against invasion plasmid antigens (Ipa) [4,6,11–13] and cell mediated immunity (CMI) [4,14–16]. However, there is no definitive evidence that these responses by themselves can be considered mechanistic mediators of protection [4]. Therefore, it is important to search for additional correlates of protection that alone or in combination can be used to predict the efficacy of candidate *Shigella* vaccines.

The appearance of ASC ~7 days after immunization suggests immune priming that may also be accompanied by the generation of B memory (B_M) cells. B_M cells are responsible for mounting a rapid anamnestic antibody response (recall response) upon re-exposure to microbial antigens and thus are considered an indicator of long-term protection induced by vaccine- or natural infection [17,18]. Methodological advances and the availability of purified antigens (including recombinant IpaB) now enable the measurement of B_M cells in cryopreserved peripheral mononuclear cell (PBMC) specimens elicited by orally administered attenuated enteric vaccines or other vaccine candidates. Using this approach we have recently demonstrated the presence of B_M cells in subjects immunized with attenuated strains of *Shigella*, *S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B and Norovirus [19–23]. Cryopreserved specimens from *Shigella* challenge studies performed in the 1990s offered a unique opportunity to identify potential immune correlates that could not be identified at that time because the technology was not available. Thus, we utilized the limited specimens remaining from those studies to measure B_M cells as well as serum antibodies in specimens collected before and after challenge, and correlated these responses with disease outcome. Our goal was to investigate correlations among pre- and post-challenge LPS- and IpaB-specific B_M and serum antibodies, as well as antibody secreting cells (ASC) with disease outcome to better define the role of specific immune responses in protection.

2. Materials and methods

2.1. Study design

We analyzed available PBMC specimens cryopreserved in liquid nitrogen and serum cryopreserved at −70 °C from the three clinical trials involving subjects challenged with wt-2457T described in the Introduction. All available PBMC specimens from 20 volunteers challenged with wt-2457T were used for B_M cell assays; 13 had prior exposure to *S. flexneri* 2a (pre-exposed group) through vaccination or experimental challenge and the remaining 7 were newly recruited healthy controls (naïve group) who had no history of prior exposure to *S. flexneri* 2a. Following thawing, an average 76% of the originally cryopreserved PBMC/vial was recovered with an average cell viability of 82% as determined by trypan blue exclusion.

PBMC were available from a pre-exposed group, comprised of three volunteers who had been previously challenged with wt-2457T (no immunizations, referred to as "veterans" in previous publications; [6,7]), and ten subjects who had been previously immunized with 4 doses of EcSf2a-2 (7×10^8 CFU). Serum samples were available from fifteen vaccinated volunteers previously immunized with 4 doses of EcSf2a-2 (7×10^8 CFU). Samples from naïve controls (PBMC: n = 7 and serum: n = 14) were also available from the same study [7]. These control subjects were healthy volunteers with no history of previous exposure to WT-2457T or EcSf2a-2. All volunteers (including the naïve subjects) were challenged with wt-2457T and samples (PBMC and sera) used in the current study were collected before (day 0) and after (day 28 days) following challenge. Informed, written consent was obtained from participants prior to enrollment in the clinical trials according to the guidelines of the Institutional Review Board of the University of Maryland.

2.2. Disease index

The design of the inpatient challenge studies is described elsewhere [6,7]. A categorical outcome-based disease index was created prior to analysis based on body temperature, daily number of bloody stools, daily number of loose stools, and total daily volume of the loose stools. The disease index assigned to each subject is the average grade of all components shown in Table 1. A dichotomous clinical outcome categorization (ill or well) was the one originally described for these clinical challenge studies (objective illness was defined on the basis