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Immune memory at 17-years of follow-up of a single dose of live attenuated hepatitis A vaccine

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ABSTRACT

Background: In recent years, hepatitis A virus (HAV) infection has declined considerably in China, associated with wide deployment of HAV vaccines and improvement in socio-economic indicators. Towards the elimination of HA in the country, we assessed the duration and characteristics of immunity conferred by the widely used, locally manufactured HAV vaccine.
Methods: This is a longitudinal cohort study that followed recipients of a live attenuated HAV vaccine 17 years after the initial administration. Blood samples were collected from participants pre- and two-week post-booster HAV vaccine dose. Serum anti-HAV antibody was measured by ELISA method. Memory B and T cells were determined by ELISPOT and Flow Cytometry assays, respectively.
Results: A robust anamnestic response was observed two-week post-challenge. Both HAV-specific memory B cell and T cells remained, and responded quickly when re-encountering HAV. The magnitude of recall responses was present, regardless of the status of the serum anti-HAV antibody pre-booster.
Conclusions: We demonstrated long-term immunity from the live attenuated HAV vaccine, including antibody persistence and immunological memory. Considering the conditions that make elimination of infectious diseases feasible, following polio, hepatitis A could be targeted for elimination in China.

1. Introduction

Hepatitis A (HA) is an acute illness caused by the hepatitis A virus (HAV). Transmission is primarily via the fecal–oral route through person-to-person contact or ingestion of contaminated food or water. The incidence of hepatitis A is strongly correlated with socioeconomic indicators [1,2]. Populations around the world are classified as having high, intermediate, low, or very low levels of HA endemicity based on seroprevalence. Until recently, China was characterized as a high endemicity country [3]. In 1992, two live attenuated hepatitis A vaccines were successfully developed in China through serial passages of the viruses in cell cultures [4]. Universal HAV immunization plus a catch-up campaign for children aged 1–15 years was considered to be feasible and HAV vaccination was integrated into the national Expanded Programme of Immunization (EPI) in 2008 [5].

During the past two decades, China has transitioned from a high to intermediate to low HA endemicity, associated with the wide deployment of HAV vaccines, economic development and changing lifestyles. The national annual incidence rate of HA declined from 56/100,000 person/years in 1991, 6/100,000 person/years in 2007 and 2/100,000 person/year in 2014; a reduction of 90% and 67% on annual incidence was observed between the period of pre- and post-introduction of HAV vaccine into the EPI [6]. Countrywide in 2014, HAV vaccine coverage was 18% among those aged 15–29 years, 64% in children 7–14 years and 91% in children 1–7 years of age [7].

With the decline in HA endemicity in China [8,9], there is the concern for the possibility of outbreaks due to waning vaccine immunity [10] and the lack of boosting from exposure to natural

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infection. It becomes critical to ensure a lifelong protection against hepatitis A virus. Clinical experience suggests that protection following vaccination might be present even in the absence of detectable anti-HAV antibodies, since memory T and B cells as well as long-lived plasma cells maintain at relatively constant numbers in the absence of the eliciting antigen for virtually a lifetime [11]. A previous study showed a good persistence of antibody following a single dose of live attenuated HAV vaccine eight years after the initial HAV vaccination [12]. In this study we assessed immunologic memory 17 years after the initial HAV vaccination and immune response following a booster dose.

2. Materials and methods

2.1. Study cohort and study design

Participants in this study were those included in a randomized trial conducted between 1996 and 1999 [13,14]. In that trial, 3,515 HAV susceptible children aged 1–12 years (mean age 5.4 years) who resided in 24 villages, were enrolled and assigned randomly to either receive a single dose of the HA vaccine or to serve as controls. To assess antibody persistence after the completion of the trial, serological follow up was performed irregularly, alternating between villages.

In this present study, recipients of HAV vaccine residing in 2 villages without either natural infection (defined as an anti-HAV antibody higher than 10,000 mIU/mL [15]) or vaccine booster (defined as either receiving traceable additional dose of HAV vaccine, or 2-fold increase of anti-HAV titers compared to that from last measurement during the 17-year serological follow-up) were selected as participants. A booster dose of HAV vaccine was administered to each participant immediately after collection of a blood sample. A second blood sample was obtained two weeks after the booster. This study was reviewed and approved by the Institutional Review Board of the Institutes of Biomedical Sciences, Fudan University. Written inform consent was obtained from each participant.

PBMC were isolated from fresh blood (10 mL) using Vacutainer cell preparation tubes (BD Biosciences, Mountain View, CA). Serum was isolated by centrifuge at 1600 rpm for 10 min, and stored at −70 °C for anti-HAV antibody assay. Peripheral blood mononuclear cells (PBMC) were isolated on Ficoll density gradients (Axis-Shield Diagnostics Ltd, Scotland) and resuspended in R-10: RPMI 1640 plus 10% FCS (heat inactivated at 56 °C for 30 min) supplemented with penicillin, streptomycin, all from Gibco (Life technology, USA). Fresh cells were used in all assays.

2.2. Vaccine and HAV antigen

The live attenuated HAV vaccine (the details of development are described elsewhere [4,16]) used both for the initial and booster doses was produced by Pukang Biotechnological Co. Hanzhou, China, with the titer of $10^{7.0}$ $TCID_{50}$ (Tissue Culture Infecting Dose).

Bulk virus (consisting of a mixture of incomplete viral particles and broken down proteins with various peptides fragments that resulted from sonication to release the live viruses replicated in the cell) with a concentration of 14 μg/mL (3000 EU/mL) that was used as antigen-specific stimulator for the measurement of T and B cells was kindly provided by the Institute of Medical Biology, the Chinese Academy of Medical Sciences.

2.3. Total anti-HAV antibodies assay

The Abbott Architect HAVab-IgG Microparticle Enzyme Immunoassay (MEIA) system (Abbott Laboratories, Chicago, USA) was employed to quantify anti-HAV antibodies in reference to W1041 anti-HAV immunoglobulin (CLB, Amsterdam, the Netherlands) [17] using non-linear regression standard curves.

2.4. Memory B cell assay: ELISPOT

The frequency of HAV Ag-specific memory B cells was measured by ELISPOT assay using ELIspot^{PLUS} for human IgG kit (MABTECH, Sweden) according to the manufacturer's instructions. Briefly, PBMC were isolated from fresh blood (10 mL) of 47 participants vaccinated 17 years ago. After pre-stimulation with a mixture of R848 and rhIL-2, PBMCs were stimulated with the prepared HAV antigens or unrelated antigen.

2.5. Memory T cell assay: Staining and flow cytometry

PBMCs were seeded into 96-well plate at a concentration of 1×10^6 cells/100 μL in R-10 and cultured at 37 °C in a 5% CO_2 incubator in the presence of HAVAg (3000 EU/mL) or medium as a negative or PMA (0.1 μg/mL, Sigma, USA) and Ionomycin (1 μg/mL, Sigma) as a positive control. Anti-CD28 antibodies (0.1 μg/mL, Miltenyi Biotec, USA) were added to all wells. Brefeldin A (0.1 μg/mL, BD Biosciences, USA) were added to all wells 6 hours before test. After 24 h of culture, the cells were washed and stained with anti-CD3 Pacific Blue^{TM} (Biolegend, San Diego), anti-CD4 APC (eBioscience, San Diego), anti-CD8a PerCp-Cy5.5 (eBioscience) and anti-CD45RO FITC (BD Biosciences monoclonal antibodies (MAbs) for 30 min in the dark on ice and washed in staining buffer (PBS containing 2% FBS), and then fixed and permeabilized using 4% paraformaldehyde (PFA, Sinopharm Chemical Reagent Co., Ltd, China) and 0.2% Triton X-100 (Genview, China) containing 2% FBS. The cells were further stained with anti-human IFN-γ APC/Cy7 (Biolegend) and IL-2 PE (eBioscience) for 60 min in the dark on ice, washed. Cell acquisitions were performed using LSR Fortessa flow cytometer (BD Biosciences, USA). For each analysis, 20,000 events were acquired in the T cell gate. Samples were first run using single fluorochrome-stained preparations for color compensation. The cytometric analysis was performed using FlowJo (TreeStar, Inc., Ashland, OR) and data are shown as% of positive cells.

To enhance the comparability of results from different measurement runs, firstly, standard operation procedure (SOPs) was established and carried out throughout the study of memory B and T cell. Secondly, isolation of PBMC and enrichment *in vitro* of all samples from 31 participants were conducted in the same runs at each time point of pre- and post-booster. Thirdly, the same positive and negative controls were implemented. Finally, to avoid the observer variations, for the measurement of memory B, ELISPORT reader was applied, while for measurements of memory T, all samples from pre- and post-booster were frozen till batch processing with flow cytometry after fixing and staining.

2.6. Statistical analysis

Anti-HAV antibody seropositive rate (SPR), geometric mean concentrations (GMCs) and their 95% confidence intervals were calculated. Antibody titers were logarithmically converted to allow assessment of GMCs. To understand the potential differences on the performances of humoral and cellular immune responses, participants were classified into anti-HAV positive and negative groups by a cutoff of 20 mIU/mL [18,19]. For continuous outcomes comparisons, the Student *t* test or the Mann–Whitney U test were undertaken, and for dichotomous outcomes, the Chi square or Fisher exact test were implemented. The GraphPad Prism 6.0 (GraphPad, San Diego, CA) was applied for statistical analysis. A p -value < .05 was considered statistically significant.

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