Vaccine 27 (2009) 4529–4536

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Parallel analysis of mucosally derived B- and T-cell responses to an oral typhoid vaccine using simplified methods

Anna Lundgren[a,*], Joanna Kaim[a], Marianne Jertborn[a,b]

[a] Göteborg University Vaccine Research Institute (GUVAX), Department of Microbiology and Immunology, University of Gothenburg, Sweden
[b] Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 30 November 2008
Received in revised form 30 April 2009
Accepted 6 May 2009
Available online 27 May 2009

Keywords:
Oral vaccination
T cell
B cell

ABSTRACT

There is a great need for simple methods for analysis of immune responses to mucosal vaccines that can be used on small blood volumes in field trials in both children and adults. We have investigated if mucosally derived B- and T-cell responses can be monitored in parallel by analysis of antibodies and T-cell effector molecules in culture supernatants from circulating blood lymphocytes obtained from orally vaccinated Swedes. Immunization with a live oral model vaccine, i.e. *Salmonella enterica* serovar Typhi Ty21a, gave rise to secretion of typhoid specific IgA and IgM antibodies from peripheral blood mononuclear cells (PBMCs) and this response could be equally well detected by ELISA and ELISPOT 7 days after vaccination. The ELISA based assay could be further simplified by using buffy coat cells, but not by using whole blood or frozen PBMCs. Vaccine induced T-cell responses appeared later than the B-cell responses, but could be detected by ELISA assessment of IFN-γ and granzymes in supernatants from antigen stimulated PBMCs 21 days after vaccination. Thus, both B- and T-cell responses could be detected using simple ELISA based assays that would be practical to use in large-scale vaccine trials.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Enteric infections are global health problems that particularly affect children in the developing world. Several promising vaccine candidates against such diseases are under development, including new and improved vaccines against enterotoxigenic *Escherichia coli* (ETEC), shigellosis and typhoid fever, or have recently come into the market, such as the new rotavirus vaccines [1]. The impact of these new vaccines on the global disease burden of enteric diseases will to a great extent depend on how effective they are in inducing protective immune responses, particularly among infants and young children in developing countries. Consequently, a great effort is currently being made to evaluate immune responses against these new mucosal vaccine candidates in children in developing countries and to improve the immunogenicity of mucosal vaccines in this important target group [2,3]. However, such evaluations are hampered by the lack of suitable methods for measurement of mucosal immune responses in younger age groups and by a lack of practical methods that are suitable for use in large-scale field trials. Most vaccine trials rely on determination of serum antibody levels, although such responses often poorly reflect mucosal immunity [4,5]. Measurements of vaccine specific antibody secreting cells (ASCs) transiently migrating in the circulation on their way to the mucosa are known to better reflect local immune responses [4,6,7]. However, the ELISPOT tests traditionally used to evaluate ASC responses are laborious, require relatively large volumes of blood, cannot be repeated using the same cell samples, and do not properly reflect mucosal booster responses. Therefore, the ELISPOT assay is less useful in large-scale vaccine trials.

Several attempts have been made to establish simplified methods to detect ASC responses. Forrest [8] developed a cell-ELISA method, in which circulating mononuclear cells were incubated in antigen-coated wells and the antibody secretion was analyzed by ELISA directly in the culture plate. Chang and Sack [9] simplified the assay further by incubating cells in the absence of antigen and storing the cell culture supernatants for later analysis of antibody content by ELISA. This so called antibodies in lymphocyte supernatants (ALS) assay makes it possible to analyze pre- and post-vaccination samples in the same test in a more practical and objective manner than by the ELISPOT or cell-ELISA methods. So far, the ALS assay has been evaluated for detection of immune responses to cholera toxin B subunit after vaccination with a licensed oral cholera vaccine, to colonization factors after immunization with an oral ETEC candidate vaccine and also to lipopolysaccharide (LPS) after vaccination with a novel typhoid vaccine [9–11]. However, to our knowledge, the assay has not yet been used in children or in large-scale vaccine trials. For these purposes, the ALS method needs to be further

* Corresponding author at: Göteborg University Vaccine Research Institute (GUVAX), Department of Microbiology and Immunology, University of Gothenburg, Box 435, 405 30, Gothenburg, Sweden. Tel.: +46 31 7866213; fax: +46 31 7866205.
E-mail address: anna.lundgren@microbio.gu.se (A. Lundgren).

0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.05.005

simplified and refined so that small blood samples could be quickly processed and used in the assay.

There is also a need for simple assays for measurement of T-cell responses. So far, such responses have mainly been determined by proliferation or cytotoxicity assays using radioactive or flow cytometric readout systems, which may not be available in all laboratories and that can be difficult to standardize. However, recent advances in detection of secreted cytokines including multiplex flow cytometric assays may facilitate the analysis of T-cell responses [12] and cytotoxic activity may now also be detected by analyzing the secretion of granzymes from activated cells using ELISA or ELISPOT [13,14].

To enable parallel evaluation of B- and T-cell responses in a Phase I/II vaccine trial, a practical approach would be to transport cell supernatants from cultures of circulating leukocytes to a central laboratory, where the presence of vaccine specific antibodies and T-cell effector molecules could be evaluated using standardized methods. In this study, we have tested this approach using the licensed oral *Salmonella enterica* serovar Typhi Ty21a vaccine as a model vaccine that is known to induce both humoral and cell mediated immunity [15]. This has included establishment of an ALS method for analysis of ASC responses and parallel evaluation of simple methods for determining T-cell derived cytokines and cytotoxic effector molecules in supernatants from antigen stimulated lymphocytes.

2. Materials and methods

2.1. Volunteers, vaccination and sample collection

Nineteen adult Swedes (median age 22 years, age range 20–42 years, 10 females) were recruited for the study. The volunteers received the oral *S. enterica* serovar Typhi Ty21a vaccine (Vivotif, Crucell BernaProducts, Switzerland) in three doses every other day. The volunteers had not previously been immunized with the Ty21a or the Vi typhoid vaccines. None of the subjects had travelled to any country where typhoid fever is endemic during the last 3 years before participating in the study.

Heparinized venous blood was collected before vaccination (day 0) and 7 and 21 days after administration of the first vaccine dose. In four of the volunteers, additional blood samples were collected on day 42. The time points for sample collection were chosen considering the results from earlier studies of ASC and T-cell responses to oral typhoid vaccines [16–18].

The study was approved by the Ethical Committee for Human Research, University of Gothenburg, and informed consent was obtained from each volunteer before participation.

2.2. Preparation of plasma and blood cells

Plasma was isolated from heparinized blood by centrifugation and stored at −20 °C.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density-gradient centrifugation on Ficoll-Paque (Pharmacia, Sweden) and counted under the microscope. Cells isolated by ficoll gradient separation are called PBMCs throughout the paper. The PBMCs were used for ALS assays as well as all T-cell methods.

For evaluation of procedures aiming to simplify the ALS assay, buffy coat cells and whole blood were also prepared and compared with PBMCs in the ALS assay. Buffy coat cells were isolated from heparinized blood by centrifugation of the blood in the collection tube at $850 \times g$ for 10 min, where after the buffy coat layer between the plasma and the erythrocytes was removed by a pipette. The buffy coat cells were then washed and the leukocytes were counted under the microscope. Whole blood was prepared by centrifugation of the blood in the collection tube at $500 \times g$ for 10 min, where after the plasma was removed and the cells were washed. The blood cells were then suspended in culture medium at 1:1, 1:2 and 1:5 dilutions in relation to the original blood volume used. Buffy coat cells and whole blood preparations were used for the ALS assay but not for the T-cell assays.

The frequencies of B cells and granulocytes in the different cell preparations were estimated by flow cytometry using forward and side scatter characteristics of the cells as well as stainings performed with the following antibodies: anti-CD3-allophycocyanin, anti-CD19-phycoerythrin, CD14-fluorescein isothiocyanate and CD45-peridinin chlorophyll protein (BD Pharmingen).

2.3. Freezing of PBMCs

PBMCs were frozen according to either a traditional protocol using cold (+4 °C) freezing medium or a more recently described protocol [19] using room tempered freezing medium. The latter protocol has been shown to give an excellent survival of cells (∼95%) and has been successfully used for evaluation of T-cell responses. The cold medium consisted of 10% DMSO and 90% fetal calf serum (FCS). The PBMCs were kept cold before diluting them in the cold medium. The cells were then immediately transferred to a pre-chilled (+4 °C) freezing container containing 2-propanol which was placed in a −70 °C freezer. The room tempered freezing medium contained a mixture of FCS and RPMI medium (Gibco). The PBMCs were kept at room temperature before mixing them with this medium and then DMSO diluted in FCS was added drop wise to the cells to a final DMSO concentration of 10%. These cells were then also frozen in a freezing container as described above. After 24 h in the −70 °C freezer, the cells were transferred to liquid nitrogen. After 1–3 weeks, the cells were quickly thawed and washed before they were counted and used in ALS assays.

2.4. Bacterial antigens

The original vaccine strain Ty21a cultured on horse blood agar plates was used for preparation of membrane proteins (MP) by sonication of followed by differential centrifugation [20]. The MP contained a large number (>20) of different proteins with different molecular sizes, as shown by gel electrophoresis, as well as LPS (8×10^5 Eu/ml), as determined by a limulus test. LPS was prepared from the same bacteria by the hot phenol water extraction method [21]. Purified *Salmonella Typhi* flagellin was kindly provided by Dr G. Dougan (The Wellcome Trust Sanger Institute, Cambridge, UK).

2.5. ALS cultures

For the ALS assay, PBMCs, buffy coat cells or whole blood preparations were cultured in Iscove's medium supplemented with 3 μg/ml L-glutamine, 50 μg/ml gentamicin and 10% FCS in flat-bottomed 96-well plates at 37 °C in 5% CO_2 (200 μl/well).

In optimization experiments, PBMCs were cultured at different concentrations (2×10^5 to 2×10^6 cells/ml) and for different times (24–96 h) and based on these results, a protocol was established where 2×10^6 PBMCs were cultured per well and supernatants were collected after 72 h. Buffy coat cells were cultured at the same cell concentration and whole blood was cultured at different dilutions (1:1, 1:2 and 1:5) and supernatants were collected after 72 h also from these cultures. All supernatants were centrifuged at $1200 \times g$ for 10 min, protease inhibitors (phenylmethylsulfonyl fluoride 350 μg/ml, soybean trypsin inhibitor 100 μg/ml) were added and the supernatants were stored at −70 °C.

Download English Version:

https://daneshyari.com/en/article/2405275

Download Persian Version:

https://daneshyari.com/article/2405275

Daneshyari.com