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A single-dose antihelminthic treatment does not influence immunogenicity of a meningococcal and a cholera vaccine in Gabonese school children

Sina Brückner [a,b], Selidji Todagbe Agnandji [a,b], Johannes Elias [c], Stefan Berberich [b], Emmanuel Bache [b], José Fernandes [b], Marguerite Massinga Loembe [a,b,d], Johanna Hass [a], Bertrand Lell [a,b], Benjamin Mordmüller [a,b], Ayola Akim Adegnika [a,b], Peter Kremsner [a,b], Meral Esen [a,b,*]

[a] Institute of Tropical Medicine, University of Tübingen, Wilhelmstraße 27, D-72074 Tübingen, Germany
[b] Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospital, BP 118 Lambaréné, Gabon
[c] Institute for Hygiene and Microbiology/NRZM, Josef-Schneider-Straße 2, 97080 Würzburg, Germany
[d] Departement de Bacterio-Virologie, Faculté de Medicine, Université des Sciences de la Santé, BP4009 Libreville, Gabon

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ABSTRACT

Background: We recently described the effect of a single-dose antihelminthic treatment on vaccine immunogenicity to a seasonal influenza vaccine. Here we report the effect of antihelminthics on the immunogenicity of a meningococcal vaccine and a cholera vaccine in primary school children living in Lambaréné, Gabon. Since infection with helminths remains a major public health problem and the influence on cognitive and physical development as well as the immunomodulatory effects are well established, we investigated if a single-dose antihelminthic treatment prior to immunization positively influences antibody titers and vaccine-specific memory B-cells.

Methods: In this placebo-controlled, double-blind trial the effect of a single-dose antihelminthic treatment prior to immunization with a meningococcal as well as with a cholera vaccine was investigated. Anti-meningococcal antibodies were assessed by serum bactericidal assay, cholera vaccine-specific antibody titers by Enzyme-linked Immunosorbent Assay (ELISA) at baseline (Day 0; vaccination), four weeks (Day 28) and 12 weeks (Day 84) following vaccination. Meningococcal and cholera vaccine-specific memory B-cells were measured at Day 0 and 84 by vaccine-specific Enzyme-linked Immunospot (ELISpot) assay. The helminth burden of the participants was assessed four weeks before vaccination (Day −28) and at Day 84 by the Merthiolate-Iodine-Formaldehyde technique.

Results: Out of 280 screened school children, 96 received a meningococcal vaccine and 89 a cholera vaccine following allocation to either the single-dose antihelminthic treatment group or the placebo group. Bactericidal antibody titers increased following immunization with the meningococcal vaccine at Day 28 and Day 84 in 68 participants for serogroup A, and in 80 participants for serogroup C. The cholera vaccine titers increased in all participants with a peak at Day 28. The number of memory B-cells increased following vaccination compared to baseline. There was no statistically significant difference in antibody and B-cell response between children receiving albendazole compared to those receiving placebo.

Conclusion: A single-dose treatment with albendazole prior to immunization had no effect on meningococcal or cholera vaccine immunogenicity in our study population.

1. Introduction

Infection with geohelminths, mainly *Ascaris (A.) lumbricoides, Trichuris (T.) trichiura* and hookworm, is a major public health problem affecting 20% of the world's population, especially in Sub-Saharan Africa (SSA). It is one of the most neglected tropical diseases with serious health, nutritional and social outcomes for affected individuals [1–3]. According to the World Health Organization (WHO) in 2014 approximately 2 billion of the world's population were infected with helminths [2], mainly children [3] and pregnant women [4]. Van den Biggelaar reported in 2004 that 46% of children, aged between 5–13 were infected with *A. lumbricoides* and 71% were infected with *T. trichiura* [5]. Three years later van Riet reported that 15% (living in a semi-urban area)

* Corresponding author at: Institute of Tropical Medicine, University of Tübingen, Wilhelmstraße 27, D-72074 Tübingen, Germany.
E-mail address: meral.esen@uni-tuebingen.de (M. Esen).

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and 55% (living in a rural area) of 7–12 year-olds were infected with *A. lumbricoides* and 12% (semi-urban area) and 64% (rural area) had a *T. trichiura* infection [6]. Chronic infection with geohelminths has an impact on health as well as on cognitive skills [7–11] and it has been shown that infection with helminths leads to altered immune responses [12].

Vaccination is one of the most effective tools to prevent infectious diseases. Nonetheless, seroconversion and therefore efficacy is variable in vaccinated individuals depending on age, environment and genetic host factors [13–15]. In addition, acute and chronic infections have an influence on vaccine outcome [16,17]. We and others have recently shown that helminth infections impair immune response to vaccination [5,6,18–22]. The WHO promotes helminth control by periodic deworming once or twice a year, depending on prevalence, as a cost-effective intervention [2,22]. Regular antihelminthic treatment through mass drug administration programs in high-risk groups like school children is considered effective for controlling the helminth infection burden, but it is not regularly applied in Gabon and other endemic countries [23]. Therefore we aimed to investigate the effect of a single-dose antihelminthic treatment on vaccine immunogenicity. Recently, we reported results of the first part of the present study, where primary school children were vaccinated with a seasonal influenza vaccine [18]. Here we report the second part of the study investigating the vaccine immunogenicity of a meningococcal and an oral cholera vaccine following a single-dose of antihelminthic treatment. The vaccines were chosen to assess whether different routes of administration (subcutaneous vs. oral) have different effects on vaccine immunogenicity in helminth infected children. Furthermore these vaccines are not part of the Expanded Program on Immunization (EPI) and we expected that no basic or low level antibody titer would be detectable if the individuals did not report recent infection with these pathogens.

2. Materials and methods

2.1. Trial design and setting

The study design is reported in detail elsewhere [18]. In brief, participants received one dose of antihelminthic treatment (albendazole 400 mg) (Micro Lab ltd, India) or placebo (Laboratories Sterop, Belgium) four weeks (Day −28) prior to vaccination with either a seasonal influenza vaccine (VAXIGRIP®, Sanofi Pasteur, season 2011/2012) intra muscularly ((i.m.) ($n = 98$) (part I)), meningococcal vaccine containing polysaccharides of *Neisseria (N.) meningitidis* group A and C (Sanofi Pasteur) subcutaneously (($n = 96$) (part Ib)) or an oral cholera vaccine containing inactivated *bacteria* and the recombinant cholera toxin B subunit ((Dukoral®, Sanofi Pasteur) ($n = 89$) (part II)) administered at Day 0. All vaccines are licensed and commercially available in Gabon. Here we focus on part Ib and II (vaccination with the meningococcal vaccine administered once at Day 0 and the oral cholera vaccine that was given twice at Day 0 and Day 7). The study took place in Lambaréné, Gabon.

Inclusion criteria were ages 6 to 10 years (primary school children), a signed informed consent form (ICF) by one of the parents or a legal guardian, no signs of chronic or acute disease upon clinical examination and no symptoms of geohelminth infection, which was assessed by the Merthiolate-Iodine-Formaldehyde (MIF)-technique. Furthermore, the participants and their legal representative were asked to reside in the study area until the end of the study.

Exclusion criteria were the participation in another clinical trial, known contraindication to antihelminthic treatment or to ingredients in one of the chosen vaccines, known immunization with one of the study vaccines; known recent meningococcal or cholera infections and any confirmed or suspected immunosuppressive or immunodeficient condition resulting from disease (e.g. malignancy, HIV infection) or immunosuppressive/cytotoxic therapy. If a child was ill or febrile at the scheduled time of the vaccination injections were postponed until convalescence.

Children who were infected with *Schistosoma (S.) haematobium* assessed by urine filtration, were excluded from the study and were treated accordingly. All parasite positive participants (including those without symptoms) received appropriate treatment after study termination.

2.2. Immunological investigations

Throughout the study period a total of 27 ml blood was collected at baseline (Day 0), Day 28 (four weeks after vaccination) and Day 84 (12 weeks after vaccination). The primary immunological endpoint of the study was functional antibody level measured by a serum bactericidal assay (SBA) for the meningococcal arm and IgG Enzyme-linked immunosorbent assay (ELISA) for the cholera arm. To assess memory B-cells (secondary immunological endpoint) a B-cell Enzyme-linked immunospot (ELISpot) assay was performed. The SBA was performed at the National Reference Center for Meningococcal Disease in Würzburg and all other investigations were performed at CERMEL and at ITM.

2.3. Assessment of anti-meningococcal antibody titers

SBA activity was assessed as described previously [24], with minor modifications. Briefly, serial dilutions of heat-inactivated sera were incubated with defined suspensions of reference strains F8238 and C11 of serogroups A and C, respectively. After 60 min, 10 µl of each well was dropped on tilted plates containing Columbia Agar with 5% sheep blood and allowed to dry. Colonies along trickle tracks were counted after overnight incubation at 35 °C and 5% CO_2 using a photographic counter (ProtoCOL, Synbiosis, Cambridge, UK). For each serum, the SBA titer represented the reciprocal of the highest dilution giving $\geqslant$ 50% killing. Titers above 4 were considered protective [25]. Titers below 4 were assigned a value of 2. A fourfold increase from pre- to post-vaccination titer was regarded as evidence of vaccine immunogenicity.

2.4. Assessment of anti-cholera antibody titers

ELISA to assess cholera-specific IgG concentrations was performed by coating the plates with a final concentration of 1 µg/ml with the *Vibrio (V.) cholerae* strain Inaba 569B (List Biological Laboratories) and incubated for 3 h at 37 °C. After washing plates were blocked over night at 4 °C. 100 µl of serum sample dilution in 10% nonfat dry milk in PBS were incubated for 1 h at 37 °C. Following another washing step, secondary antibody (anti-human IgG γ-chain specific peroxidase conjugate (SIGMA, Germany)) was added. To visualize the bound antibodies, the plate was incubated for 20 min in the dark with a color solution (TMBONE, KemEnTech). The reaction was stopped using 2 M H_2SO_4. The plate was measured at 450 nm (620 nm reference) with an ELISA reader. The OD values were converted using linear regression of a serial dilution of standards into concentrations using the statistical software environment R v2.9.0 [26].

2.5. Vaccine-specific memory B-cell ELISpot

Antibody secreting cells (ASCs) representing memory B-cells were assessed with ELISpot assay as described elsewhere [18] with slight modifications which were the following: Plates were coated directly with 5 µg/ml of the meningococcal vaccine or 10 µg/ml of

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