The effects of BCG-vaccination on the innate immune response and immunoparalysis in healthy volunteers. Two proof-of-principle studies.

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Immune disorders NEC
Study type	Interventional

Summary

ID

NL-OMON40604

Source
ToetsingOnline

Brief title
Effects of BCG on immune response

Condition

- Immune disorders NEC
- Bacterial infectious disorders

Synonym
bacterial bloodstream infection, sepsis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Sint Radboud
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: BCG, Immunoparalysis, LPS, Sepsis

Outcome measures

Primary outcome

Single endotoxemia:

the primary study endpoint is the differences in LPS-induced plasma concentration of TNF-α following endotoxemia, between γ-irradiated BCG-vaccined subjects and placebo-treated controls.

Repeated endotoxemia:

the primary study endpoint is the difference in the LPS-induced TNF-α concentration following the first and second endotoxemia, between γ-irradiated BCG-vaccined and placebo-treated control subjects.

Secondary outcome

Secondary study parameters include various other inflammatory cytokines, including IL-6 and IL-10, the ex vivo production of inflammatory mediators by stimulated leukocytes, the phenotype of circulating monocytes, inflammatory transcriptional pathways (by use of qPCR/microarrays), epigenetic changes in leukocytes including H3K4 trimethylation, illness score, mean arterial pressure, heart rate and temperature.

Study description

Background summary

Sepsis is a major medical challenge associated with a high mortality rate. Release of pro-inflammatory mediators can result in hemodynamic instability, coagulation abnormalities and end-organ dysfunction. Previous strategies have aimed to treat sepsis by inhibition of pro-inflammatory mediators, however, most of these approaches have failed. This might be due to the fact that the majority of septic patients do not succumb to the initial pro-inflammatory *hit*, but die at a later time-point in a pronounced immunosuppressive state. This so-called *immunoparalysis*, which renders patients extremely vulnerable to secondary infections, results from the triggering of counter-regulatory anti-inflammatory pathways along with the pro-inflammatory response, already starting in the beginning of sepsis. Immunoparalysis is increasingly being recognized as the overriding immune dysfunction during sepsis. As a consequence, reconstitution of immunocompetence is now emerging as a new and promising therapeutic target to improve outcome in sepsis patients.
Bacille Calmette-Guérin (BCG) is one of the most commonly administered vaccines worldwide. In addition to protection against tuberculosis, evidence suggests that BCG immunization has a number of additional beneficial non-specific immunological effects, hereby protecting against infections with pathogens other than tuberculosis. The underlying immunologic mechanisms are not fully elucidated. Recently it was demonstrated that monocytes can be functionally reprogrammed to an enhanced and lasting phenotype after vaccination with BCG. Production of pro-inflammatory cytokines by monocytes isolated from volunteers after BCG vaccination, was found to be enhanced upon ex vivo stimulation with non-related pathogens, even months after BCG vaccination. The observed effects are proposed to be due to modulation of the innate immune system in a process called *trained immunity*. Upon stimulation with a pathogen, the innate immune system becomes primed and is able to react faster and more efficient to a secondary (and non-related) stimulus, even months later. Monocyte *training* was shown to rely on epigenetic reprogramming, namely increased methylation of histone 3 at lysine 4 (H3K4me3) at the level of cytokine and TLR4 gene promoter regions.

Considering these potentiating effects of BCG on innate host defense, it could be a viable treatment option for sepsis-induced immunoparalysis. However, the effects of BCG vaccination on the innate immune response in humans have hitherto only been shown ex vivo. It has yet to be established whether these findings can be extrapolated to the human in vivo situation, because previous data from our group indicates that ex vivo measurements do not accurately reflect the in vivo situation. The human endotoxemia model, in which healthy volunteers receive lipopolysaccharide (LPS) derived from Escherichia coli, is widely used to study the effects of systemic inflammation in humans in vivo and

is considered a safe and highly reproducible method to investigate the innate immune response. Furthermore, LPS administration results in a hyporesponsive state towards a second LPS administration called *endotoxin tolerance*, which resembles sepsis-induced immunoparalysis, and can thus be used as a model to investigate therapeutic interventions to reverse this condition.

The intended target group for this novel therapy, sepsis patients, are immunocompromised. Therefore, use of a live attenuated vaccine such as BCG could present a risk of disseminated mycobacterial infection. Therefore, we will use γ-irradiated (inactivated) BCG vaccine in this study. Recent, yet unpublished results of the group of Prof. Netea have shown that the effects of γ-irradiated BCG on monocyte training are comparable to those of the live vaccine.

Study objective

In the present study, we first want to investigate whether BCG-vaccination enhances the innate immune response in humans in vivo during human endotoxemia. In the second experiment we want to investigate whether BCG-vaccination can reverse the tolerant state observed upon a second LPS administration. Our goal is to ultimately translate our results into clinic applications to reverse for example sepsis-induced immunoparalysis.

Primary objective:
1. Single endotoxemia
To determine the effects of γ-irradiated BCG-vaccination on the in vivo innate immune responses induced by human endotoxemia. This will be determined by measuring plasma levels of various pro- and anti-inflammatory cytokines and assessing the difference in the Lipopolysacharide (LPS)-induced cytokine response between γ-irradiated BCG-vaccined subjects and placebo-treated control subjects.

2. Repeated endotoxemia
To determine the effects of γ-irradiated BCG-vaccination on endotoxin tolerance induced by human endotoxemia. This will be determined by measuring plasma levels of various pro- and anti-inflammatory cytokines and assessing the difference in the LPS-induced cytokine response following the first and second endotoxemia, between γ-irradiated BCG-vaccined and placebo-treated control subjects.

Secondary Objective(s): There are 5 secondary objectives:
1. To determine the effects of γ-irradiated BCG-vaccination on ex vivo responsiveness of leukocytes to various inflammatory stimuli.

2. To determine the effects of γ-irradiated BCG-vaccination on the phenotype of circulating monocytes (e.g. expression pattern of cell-surface receptors by use of flow cytometry).

3. To determine the effects of γ-irradiated BCG-vaccination on inflammatory transcriptional pathways (by use of qPCR/microarrays).

4. To determine the effects of γ-irradiated BCG-vaccination on epigenetic changes, including H3K4 trimethylation, in circulating immune cells.

5. To determine the effects of γ-irradiated BCG-vaccination on LPS-induced clinical symptoms (illness score) and hemodynamic/temperature changes.

Study design

Study design
1. Single endotoxemia
A randomized double-blind placebo-controlled pilot study in healthy human volunteers during experimental endotoxemia.
In this pilot study, we will enrol 20 subjects. On day 1, 10 subjects will receive γ-irradiated BCG-vaccination and 10 subjects will receive placebo. On day 6, all subjects will undergo experimental endotoxemia.

2. Repeated endotoxemia
A randomized double-blind placebo-controlled pilot study in healthy human volunteers during repeated experimental endotoxemia. This study will be performed after study 1 has been completed, and the results of study 1 have been discussed with the ethical committee. In this pilot study, we will enrol 16 subjects. All subjects will undergo experimental endotoxemia on day 1. On day 3, 8 subjects will receive γ-irradiated BCG-vaccination and 8 subjects will receive placebo. On day 8, all subjects will undergo experimental endotoxemia for the second time.

Intervention

Single endotoxemia:
all subjects (n=20) are double-blind randomized to receive either γ-irradiated BCG-vaccination (n=10) or placebo (NaCl 0.9% subcutaneously, n=10) on day 1. On day 6, all subjects will undergo experimental endotoxemia (LPS derived from E coli O:113, 1 ng/kg).

Repeated endotoxemia:
all subjects (n=16) will undergo experimental endotoxemia on day 1 (LPS derived from E coli O:113, 2 ng/kg). On day 3, subjects will receive either γ-irradiated BCG-vaccine (n=8) or placebo (NaCl 0.9% subcutaneously, n=8) in a randomized, double-blind manner. On day 8, all subjects will undergo experimental endotoxemia for the second time (LPS derived from E coli O:113, 2 ng/kg).

Study burden and risks

All subjects will visit the hospital for a screening visit in which a medical interview and physical examination will be carried out (30 minutes).

BCG is one of the world*s most widely used vaccines and is well-tolerated in healthy volunteers. Local side-effects are frequently seen but do not cause serious harm. To avoid serious systemic adverse events, ie disseminated Mycobacterium Bovis infection, we choose to use γ-irradiated (inactivated) BCG substrate. Local side-effects are expect to be less pronounced compared to live vaccine. Seroconversion and thus positivity to Mantoux testing, has been described in 41.8% after vaccination with alive BCG, reduced to only 21.2% after 10 years. In case of serconversion, Mantoux cannot be used as a diagnosticum for tuberculosis any longer. However, other diagnostics such as Quantiferon and chest x-ray can still be used.

During endotoxemia, volunteers will be monitored on the research unit of our intensive care and receive an arterial line to facilitate blood pressure monitoring and blood sampling. The arterial line will be placed ultrasound guided and under local anaesthesia using 2% lidocaïne. Furthermore, a venous cannula will be placed for the administration of fluids and LPS. The administration of LPS induces flu-like symptoms for approximately 4-6 hrs. This model of systemic inflammation has been applied for 10 years in our department and thousands of subjects in various research centres in the world have participated in experimental endotoxemia trials. LPS administration is considered safe and no long-term effects have ever been documented. Lipopolysacharide 2 ng/kg is the most widely used dose in human endotoxemia research, although higher doses (for example 4 ng/kg) have also been used with no documented long term effects. In 18 human endotoxemia trials performed in our centre to date (see IMPD), we have used 2 ng/kg. In one trial (CMO 2006/166), we administered increasing LPS dosages on 5 consecutive days (0.2, 0.5, 1, 2, and 2 ng/kg). Because we hypothesize that BCG-vaccination will potentiate the immune response following human endotoxemia, we will administer only half the dose of LPS normally used, namely 1 ng/kg, in our first, single-endotoxemia study. In the second study evaluating immunoparalysis, 2 ng/kg will be used, since treatment with BCG will take place after the first LPS administration, which will result in a blunted response upon a second LPS administration. It is to be expected that BCG will at most restore the response to a second LPS administration to levels comparable with those observed during the first LPS administration, but not potentiate it further. 2 ng/kg LPS was also used in a previous trial (CMO 2011/105) in which LPS was administered twice, where very potent compounds (IFN-γ and GM-CSF) were used to reverse the tolerant state after the first LPS administration. In this trial, IFN-γ was most effective in restoring the immune response upon a second LPS administration but did not potentiate it beyond the level observed after the first LPS administration.

At the Radboud University Medical Centre, over 370 volunteers have received more than 445 injections of LPS. Therefore, there is sufficient experience

with this model at this centre. LPS administrations will be carried out in a consecutive manner. Furthermore, randomization will be carried out in a manner guaranteeing that LPS will not be administered to more than one person with the same intervention (BCG-vaccination or placebo) on one day. A physician or nurse will be present during the LPS experiment at all times, and subjects will be continuously monitored (heart rate, blood pressure, oxygen saturation). In total, a maximum of 600 ml blood will be drawn during the study, which is comparable to previous studies and never resulted in adverse events. Subjects will not benefit directly from participation to the study. A subject fee is provided.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

- Written informed consent
- Age >=18 and <=35 yrs
- Male
- Healthy

Exclusion criteria

- Use of any medication
- History of BCG-vaccination
- Vaccination other than BCG, within 3 months prior to study or within study period
- Smoking
- Previous spontaneous vagal collapse
- History of atrial or ventricular arrhythmia
- (Family) history of myocardial infarction or stroke under the age of 65 years
- Cardiac conduction abnormalities on the ECG consisting of a 2nd degree atrioventricular block or a complex bundle branch block
- Hypertension (defined as RR systolic > 160 or RR diastolic > 90)
- Hypotension (defined as RR systolic < 100 or RR diastolic < 50)
- Renal impairment (defined as plasma creatinin >120 µmol/l)
- Liver enzyme abnormalities or positive hepatitis serology
- Medical history of any disease associated with immune deficiency
- CRP > 20 mg/L, WBC > 12x109/L, or clinically significant acute illness, including infections, within 4 weeks before endotoxin administration
- Participation in a drug trial or donation of blood 3 months prior to the LPS challenge
- Use of recreational drugs within 21 days prior to experiment day
- Recent hospital admission or surgery with general anaesthesia (<3 months)
- Disagreement of participant with informing general practitioner of participation in this study

Study design

Design

Study phase:	3
Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)

Control:	Placebo
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	24-04-2014
Enrollment:	36
Type:	Actual

Medical products/devices used

Product type:	Medicine
Brand name:	γ-Irradiated BCG vaccine (BCG-Vaccin SSI [Nederlands Vaccin Instituut]) Danish strain 1331.

Ethics review

Approved WMO Date:	13-01-2014
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	28-04-2014
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
EudraCT	EUCTR2013-005520-42-NL
CCMO	NL47558.091.13

Study results

Date completed:	01-11-2015
Actual enrolment:	20