The effects of human endotoxemia on the functional capacity of hematopoietic stem and progenitor cells

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Primary objective: - To characterize hematopoietic progenitor cells in human bone marrow before and after endotoxemia (by evaluating hematopoietic lineage differentiation and functional capacity). Secondary Objectives: - To determine the effects of...

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

Summary

ID

NL-OMON45383

Source
ToetsingOnline

Brief title
LPS-BM study

Condition

- Immune disorders NEC
- Bacterial infectious disorders

Synonym
bacterial bloodstream infection, Sepsis

Research involving
Human

Sponsors and support

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

Intervention

Keyword: bone marrow, endotoxemia, endotoxin tolerance, HSPC

Outcome measures

Primary outcome

Characterization of human hematopoietic stem and progenitor cells before and after endotoxemia determined by:

1) Hematopoietic lineage differentiation (composition and cellularity) of the bone marrow compartment by FACS

2) Functional capacity by means of ex vivo cytokine release (including but not limited to IL-1β, TNF-α, IL-6, IL-10, IFN-γ) upon stimulation with PAMPs (e.g. LPS) and pathogens (e.g. S. aureus, C. albicans, M. tuberculosis, S. pneumonia)

Secondary outcome

• Ex vivo cytokine release of alveolar macrophages upon stimulation

• Transcriptome of hematopoietic stem and progenitor cells, blood leukocytes, and alveolar macrophages

• Epigenome of hematopoietic stem and progenitor cells, blood leukocytes, and alveolar macrophages

• Cellular metabolism of hematopoietic stem and progenitor cells, blood leukocytes, and alveolar macrophages

• Gene polymorphisms relating to innate immune function (e.g. autophagy ATG2b and ATG5 SNPs)

• Life span and transit times of different subsets of leukocytes and their progenitors in human bone marrow (mitotic and post-mitotic pool) and the blood

compartment

• Circulating cytokine concentrations upon endotoxemia

• Vital parameters during endotoxemia (mean arterial pressure, heart rate and

temperature)

• Illness score during endotoxemia

Study description

Background summary

Sepsis is a major medical challenge characterized by a systemic inflammatory response, and is associated with a high mortality rate and increasing incidence. The immune response in sepsis is highly variable between patients and can comprise both a hyperinflammatory and a immunosuppressive state. The experimental human endotoxemia model is widely used to study on the effects of systemic inflammation in vivo in humans, because it bears hallmarks of both the hyperinflammatory and a immunosuppressive state of sepsis, the latter exemplified by the development of endotoxin tolerance. To date, mechanistic research into systemic inflammation in humans has been limited to the peripheral blood compartment, as tissue-residents cells are not easily available for harvesting. A discrepancy in duration of ex vivo and in vivo endotoxin tolerance of circulating leukocytes, and the lack of correlation between ex vivo and in vivo responses to endotoxin administration in humans, suggest that immune cells in other compartments, rather than circulating blood leukocytes, are important effectors of the in vivo inflammatory response. The function of bone marrow cells in the in vivo inflammatory response is currently unknown. Hematopoietic stem and progenitor cells in bone marrow express Toll-like receptors and thereby are assumed to have immunomodulatory functions. Macrophages derived from human hematopoietic stem and progenitor cells exhibit reduced inflammatory cytokine production after being exposed to a TLR2 agonist prior to macrophage development. These data suggest a pivotal role for the bone marrow compartment in regulation of the inflammatory response. In this study, we will investigate whether LPS induces changes in function of bone marrow cells and their downstream effector cells. Furthermore, we will also investigate other compartments such as the blood and the lungs. To comprehensively investigate underlying mechanisms behind functional changes in these cell types, we will use state-of-the-art systems biology techniques, including transcriptomics, epigenetics, and metabolomics.

Study objective

Primary objective:
- To characterize hematopoietic progenitor cells in human bone marrow before and after endotoxemia (by evaluating hematopoietic lineage differentiation and functional capacity).

Secondary Objectives:
- To determine the effects of endotoxemia on the functional capacity of alveolar macrophages in the sputum.
- To determine whether endotoxemia induces changes in the transcriptome (inflammatory transcriptional pathways) of human hematopoietic stem and progenitor cells , blood leukocytes, and alveolar macrophages.
- To determine whether endotoxemia induces changes in the epigenome (complement of chemical compounds altering gene expression) of human stem and hematopoietic progenitor cells, blood leukocytes, and alveolar macrophages.
- To determine whether endotoxemia induces changes cellular metabolism of hematopoietic stem and progenitor cells, blood leukocytes, and alveolar macrophages.
- To determine the correlation of gene polymorphisms relating to innate immune function (e.g. autophagy ATG2b and ATG5 SNPs) with immune function and induction of endotoxin tolerance.
- To determine the life-span and transit times of different subsets of leukocytes and their progenitors in the bone marrow of humans at homeostasis and during endotoxemia.

Study design

An explorative randomized placebo-controlled pilot study in 12 healthy male volunteers.

The study takes place at the research unit of the intensive care department of the Radboud University Medical Center, Nijmegen. The duration of the study period for each individual subject will be three weeks. Volunteers will be recruited and are subjected to a medical examination (including interview, medical history and physical examination). After medical approval and informed consent subjects will be randomly allocated to the LPS group (n=8) or to the placebo group (n=4).

On first challenge day, all subjects will be challenged with a bolus LPS of 2ng/kg (n=8) or a placebo solution (n=4). On second challenge day, only the LPS group (n=8) will be subjected to a second LPS challenge to determine the extent of in vivo endotoxin tolerance.

To characterize the hematopoietic stem and progenitor cells, subjects will undergo a bone marrow examination three times; one week before (baseline), 4 hours after, and 7 days after the first challenge. Furthermore, blood will be withdrawn and sputum (containing alveolar macrophages) will be collected. In addition, deuterium glucose will be used to label mitotic cells to track

downstream inflammatory leukocytes.

Intervention

This study investigates the effects of experimental endotoxemia on the systemic inflammatory response and endotoxin tolerance in different compartments of the human body (bone marrow, blood, and lungs). As such, LPS will be used as a challenge agent to induce systemic inflammation. No investigational (medication) treatment is given. The eight subjects in the LPS group will be challenged with LPS twice, on day 8 and day 15. The four subjects in the placebo group will receive a placebo challenge on day 8 and they won*t be challenged on day 15. There is no need for a blinded procedure as the effects of LPS injection will be profound.

Study burden and risks

Subjects will visit the research unit a total of 6 times; for screening, first labeling day, first bone marrow examination, second labeling day, first challenge day, and second challenge day. Volunteers will be recruited and are subjected to a medical examination (including interview, medical history and physical examination). The administration of a lipopolysaccharide induces flu-like symptoms for approximately 3-4 hrs. This model of systemic inflammation has been applied for more than 10 years in our department and thousands of subjects worldwide have participated in endotoxemia trials. During the LPS challenge, subjects will be under constant supervision of an experienced physician with continuous monitoring of blood pressure and heart rate. The endotoxemia protocol and associated risks are identical to earlier endotoxemia studies performed in our institute. Bone marrow examinations will be performed by a highly experienced physician assistant of the hematology department. The bone marrow examinations in a previous study were very well tolerated by the subjects. A maximum of 500 ml blood will be drawn during the study, which is comparable to previous studies and never resulted in adverse events. The labeling of leukocytes is safe, non-invasive, and the group of prof. Koenderman is very experienced in using this technique. The subjects will not benefit directly from participation to the study. This study will yield a comprehensive insight into inflammatory signaling in the bone marrow and will thereby improve our understanding of systemic inflammation, endotoxin tolerance, and sepsis, possibly revealing new therapeutic targets to improve sepsis outcome. The risks to the subjects in this study is classified as a *negligible risk* (low risk on minor harms). 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 (as confirmed by medical history, examination, ECG, blood sampling)

Exclusion criteria

• Use of any medication
• Smoking
• History or signs of atopic syndrome (asthma, rhinitis with medication and/or eczema)
• Known anaphylaxis or hypersensitivity to the non-investigational products or their excipients.
• History or signs of hematological disease (bone marrow dysfunction):
• Thrombocytopenia (<150*10^9/ml) or anemia (hemoglobin < 8.0 mmol/L)
• Abnormalities in leukocyte differential counts

• History, signs or symptoms of cardiovascular disease, in particular:
• Previous spontaneous vagal collapse
• History of atrial or ventricular arrhythmia
• Cardiac conduction abnormalities on the ECG consisting of a 2nd degree atrioventricular block or a complete left 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 creatinine >120 µmol/l)
• Liver enzyme abnormalities (above 2x the upper limit of normal)
• Medical history of any disease associated with immune deficiency
• CRP > 20 mg/L, WBC > 12x109/L or < 4 x109/L, or clinically significant acute illness, including infections, within 3 weeks before labeling day
• Previous (participation in a study with) LPS administration
• Any vaccination within 3 months prior to labeling day
• Participation in a drug trial or donation of blood 3 months prior to labeling day
• Recent hospital admission or surgery with general anesthesia (<3 months to labeling day)
• Use of recreational drugs within 21 days prior to labeling day
• Inability to personally provide written informed consent (e.g. for linguistic or mental reasons) and/or take part in the study.

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Open (masking not used)
Control:	Placebo
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	24-01-2018
Enrollment:	12
Type:	Actual

Ethics review

Approved WMO Date:	17-07-2017
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	26-09-2017
Application type:	Amendment
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	15-09-2020
Application type:	Amendment
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
CCMO	NL61136.091.17