NET formation by neutrophils

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To gain a better understanding of NET formation by human neutrophils and the molecular mechanisms underlying this process.We would like to gain insight in:* The differences in NET formation upon the exposure of neutrophils to different stimuli.* The...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Autoimmune disorders
Study type	Observational invasive

Summary

ID

NL-OMON46357

Source
ToetsingOnline

Brief title
NETs

Condition

- Autoimmune disorders

Synonym
Autoimmune diseases

Research involving
Human

Sponsors and support

Primary sponsor: Radboud Universiteit Nijmegen
Source(s) of monetary or material Support: Ministerie van OC&W,STW (NWO-TTW)

Intervention

Keyword: Bloodcell isolation, Immune system, NETosis, Neutrophils

Outcome measures

Primary outcome

The data obtained from in vitro experiments will be the outcome of the study.

Secondary outcome

Not applicable

Study description

Background summary

NETs, which are generated during a process termed NETosis, represent the most recently described weapon in the arsenal of neutrophils to combat pathogens. In the process of NETosis, the neutrophil ejects its chromatin, which is decorated with antimicrobial proteins. The resulting neutrophil extracellular trap (NET) is a web-like structure composed of DNA and proteins that can catch invading pathogens and prevent them from spreading through the rest of the body. The formation of NETs was described for the first time in 2004, and since then a lot of research has been performed to obtain more insight in the process of NET formation, although still many questions remain unanswered. With the current study we would like to increase our fundamental knowledge of NET formation.

NETosis can be well studied in in vitro experiments, using (human) neutrophils isolated from freshly drawn blood of healthy individuals. The isolated neutrophils are seeded in plastic well-plates or petri-dishes and subsequently stimulated with different inducers of NETosis. In the literature, various stimuli have been described and because these are not uniformly used by different research groups it is often hard to compare existing data. Importantly, all these stimuli appear to induce NETosis via distinct mechanisms. We aim to shed more light on the diversity of the corresponding pathways and the cellular and molecular features that characterize them.

The characterization of NET formation is clinically relevant since NETosis has been implicated in many diseases since its first description in 2004. It was soon anticipated that the exposure of the immune system to DNA and other intracellular components might lead to an autoimmune response. Subsequently, several investigations have addressed the influence of NETs on autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Furthermore, the antimicrobial proteins associated with NETs may also be harmful to host tissue if not properly cleared. Finally, NETs were shown to obstruct blood vessels and induce clotting, and might therefore also be

involved in thrombotic diseases. A better fundamental understanding of NETosis might aid in the development of treatment for the above-mentioned disease states, for example by designing drugs that inhibit NET formation, or drugs that efficiently degrade NETs to prohibit clogging and tissue injury.

Study objective

To gain a better understanding of NET formation by human neutrophils and the molecular mechanisms underlying this process.
We would like to gain insight in:
* The differences in NET formation upon the exposure of neutrophils to different stimuli.
* The involvement of protein-modifying enzymes in NET formation
* The citrullinated protein content of NETs
* The intracellular calcium and ROS levels during NET formation

Study design

This study is based on the isolation of neutrophils from blood donated by healthy volunteers. NET formation will be studied in in vitro experiments. Blood samples will be collected via venipuncture after signed informed consent; the volume will be between 10 and 40ml. The exact amount depends on the nature of the planned experiment and the amount of neutrophils that is needed to perform it. Donors are not followed over time or subjected to other procedures than donating blood. This research will not yield findings that maybe stressful or clinically relevant to the donors since the only clinical parameter that will be checked are cell counts. Since these cell counts are different anyway between different donors, no meaningful conclusions can be drawn upon the outcome of these numbers. This study does not entail minors or incapacitated adults.

We have chosen for this study design instead of purchasing so-called buffy coats of Sanquin, since neutrophils only live for one day and products of Sanquin are often stored overnight. Moreover, we only need relatively small amounts of blood to be able to perform experiments. For reliable NET formation experiments, it is important to collect the material via standardized procedures, which can be best controlled when the blood is obtained as freshly as possible.

After oral informed consent, the healthy donor will undergo venipuncture to donate 10 to 40 ml blood, which is collected in EDTA anti-coagulation tubes. The venipuncture is performed by nurses from the departments of Neurology at the RadboudUMC. Neutrophils are isolated from the donated blood according to Standard Operating Procedures and subsequent in vitro experiments will be performed on the day of donation. After the experiments are finished, all materials will be discarded; biomaterials involved in this study will not be

stored.

Study burden and risks

Risks and burdens: Risk is negligible and the burden is minimal. The most common risks related to drawing blood from the subject*s arm are brief pain and/or bruising. Infection, excess bleeding, clotting or fainting is also possible but unlikely.
Benefits: There will not be direct benefit. However, participation of subjects can help researchers to gain knowledge.

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

Healthy volunteers between 18 and 65 years old. Specific characteristics such as sex and ethnic background are irrelevant.

Exclusion criteria

Subjects who are not healthy, not feeling well or older than 65 or younger than 18

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	24-09-2018
Enrollment:	100
Type:	Actual

Ethics review

Approved WMO Date:	09-04-2018
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	30-08-2018
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	NL64174.091.18