Monocyte dysfunction in Multiple Sclerosis (MS)

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1). Evaluate the expression of the genes encoding the enzymes that modify histones and DNA during monocyte differentiation in MS.2). Consequences of monocyte dysfunction for migration and cytokine/chemokine production.

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

Summary

ID

NL-OMON36307

Source
ToetsingOnline

Brief title
Monocyte dysfunction in Multiple Sclerosis (MS)

Condition

- Autoimmune disorders
- Central nervous system infections and inflammations
- Arteriosclerosis, stenosis, vascular insufficiency and necrosis

Synonym
Multiple Sclerosis

Research involving
Human

Sponsors and support

Primary sponsor: Vrije Universiteit Medisch Centrum
Source(s) of monetary or material Support: Stichting MS Research

Intervention

Keyword: Epigenetics, Inflammation, Monocytes, Multiple Sclerosis

Outcome measures

Primary outcome

NA

Secondary outcome

NA

Study description

Background summary

We have developed an interest over the years in monocyte dysfunction in vascular disease and multiple sclerosis (MS), both recognized by inflammation. In atherosclerosis particularly monocytes navigate to sites of endothelial damage, adhere to the vessel wall and transmigrate the endothelial cell layer to infiltrate the underlying tissue. Upon transendothelial migration, monocytes differentiate into functionally defined subsets i.e. M1-macrophages (inflammatory) or M2-macrophages (anti-inflammatory) and into dendritic cells, depending on the stimulus they encounter in the local micro-environment of the vessel wall (Wierda et al., 2010). Interestingly, the infiltration of monocytes within the vasculature has a paradoxical dual role: continuous and prolonged infiltration may drive chronic inflammation, which results in occlusion of the vessel wall (stenosis), whereas transient recruitment promotes collateral artery growth and tissue repair. Recent insights into the pathogenesis of MS reveal parallels to the processes that occur in vascular diseases. Stenosis affecting the cerebrospinal venous segment hampers venous flow, which could trigger an inflammatory response. Subsequently, monocytes are then attracted by and transmigrate the inflamed cerebrovascular endothelium, where they further differentiate into defined subsets of macrophages/microglia in the micro-environment of the brain. During monocyte differentiation remodeling of the chromatin structure by modifications of histones and DNA is essential, because they control transcription programs of genes that determine lineage-specificity and cell activation. It is hypothesized that besides the contribution of the inflamed (cerebro)vascular endothelium, also inflammation-induced changes in differentiation-fate and function of monocytes contributes to disease. Goal of this pilot grant application therefore is to investigate disease-associated alterations in the expression of epigenetic

effectors in MS, and the bearing this has on monocyte differentiation and transendothelial cell migration. A better understanding of the molecular mechanisms responsible for monocyte differentiation and trafficking from the periphery into the diseased CNS will ultimately help to intervene in these processes in MS.

Study objective

1). Evaluate the expression of the genes encoding the enzymes that modify histones and DNA during monocyte differentiation in MS.

2). Consequences of monocyte dysfunction for migration and cytokine/chemokine production.

Study design

1). Evaluate the expression of the genes encoding the enzymes that modify histones and DNA during monocyte differentiation in MS.

To evaluate the mRNA expression characteristics of most of the currently known lysine acetyltransferases, lysine deacetylases and sirtuins, lysine methyltransferases and lysine demethylases, and also of DNA methyltransferases in cultured cells of human origin, we have developed a real-time RT-PCR array allowing the specific and quantitative monitoring of these expression levels.

For the purpose of these investigations, CD14+ monocytes will be isolated from Fycoll gradient separated peripheral blood mononuclear cells (PBMCs) by magnetic cell sorting (MACS® Technology) with the use of CD14+ Macs beads (Miltenyi Biotec). For polarization into macrophage type 1 (M1, pro-inflammatory), cultured CD14+ monocytes will be exposed to 50 U/ml recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) and for polarization into macrophage type 2 (M2, anti-inflammatory) to 50 ng/ml macrophage colony stimulating factor (M-CSF) for 5 days. For polarization into immature dendritic cells (iDCs), cultured CD14+ monocytes will be exposed to 1000 U/ml (GM-CSF) and 500 U/ml IL-4 for 5 days. For activation, polarized CD14+ monocytes subsequently will be exposed to 100 ng/ml LPS for 2 days.

mRNA will be isolated from unstimulated monocytes and from monocytes after polarization and/or activation, which will be converted into cDNA for real-time reverse transcriptase (RT)-PCR on an ICycler IQ system using the IQ SYBR-Green Supermix. The culture media of the polarized monocytes will be stored for analysis for levels of secreted cytokines/chemokines (see below).

2). Consequences of monocyte dysfunction for migration and cytokine/chemokine production

Freshly isolated CD14+ monocytes obtained from PBMCs of MS patients will be analyzed phenotypically by flowcytometry for the expression of the chemokine receptors CCR5, CX3CR1 and CXCR4. Next, the migratory capacity of these monocytes will be tested in chemotaxis assays in the response to CCL5 and CCL3 (ligands for CCR5), CX3CL1 (ligand for CX3CR1), CXCL12 (ligand for CXCR4) and C5a (as control) in a 48-well microchemotaxis chamber as previously explored for microglial cells (Kuipers et al., Glia 2006). Results will be compared with CD14+ monocytes isolated from PBMCs of healthy control individuals.

To functionally examine whether the chemotaxis has altered the expression characteristics of the epigenetic effectors, the migrated cells will be collected and profiled as detailed in section 1. Results will be compared with non-migrated cells.

For the determination of alterations in the secretion levels of cytokines and chemokines during monocyte polarization in MS, collected culture media of polarized and activated monocytes as detailed above will be analysed for production of a variety of cytokines/chemokines by luminex technology with use of a human 27-plex panel BioPlex cytokine assay kit (BioRad).

Statistical significance of the obtained results in sections 1 and 2 will be determined by Student t-test or Mann-Whitney U-test for group variables. Correlation will be determined by Pearson*s rank order or by Spearman*s rank order correlation.

Study burden and risks

Collection of PBMCs (3 tubes of 10 mls) is a very benign burden with hardly any risk.

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

Primary progressive MS, secondary progressive MS and Relapsing/Remitting MS

Exclusion criteria

Not applicable

Study design

Design

Study type: Observational non invasive

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

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	09-06-2011
Enrollment:	40
Type:	Actual

Ethics review

Approved WMO Date:	29-03-2011
Application type:	First submission
Review commission:	METC Amsterdam UMC

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	NL33219.029.11