CONTRIBUTION OF THE UREMIC MILIEU TO THE PRO-INFLAMMATORY MONOCYTIC PHENOTYPE

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Introduction

Patients with chronic kidney disease (CKD) are in a **chronic state of micro-inflammation**:

- Associated with accelerated cardiovascular disease → leading cause of death in CKD.
- **Monocytes** play an important role in chronic inflammation and have a function in every stage of atherogenesis.

Monocytes consist of three **subpopulations**, based on their surface markers expression:

1. Classical monocytes **CD14^{++} CD16^{-}**
2. Intermediate monocytes **CD14^{++} CD16^{+}**
   → most **pro-inflammatory** and **atherogenic**
   → **increased** in number in both **CKD** and **hemodialysis** patients
3. Non-classical monocytes **CD14^{+} CD16^{++}**

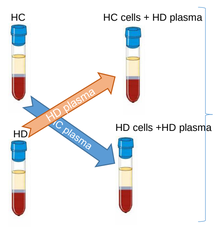
Aim

Analyze the **role of the uremic milieu** and **specific uremic toxins** in **monocyte differentiation** towards the **pro-inflammatory phenotype.**

Materials & Methods

Exchange of plasma: control vs. hemodialysis

- Sodium citrate whole blood
- Healthy control (HC) and hemodialysis (HD) patient: blood group matched
- Sham-exchange and real exchange of plasma
- Incubation for 24h at 37°C

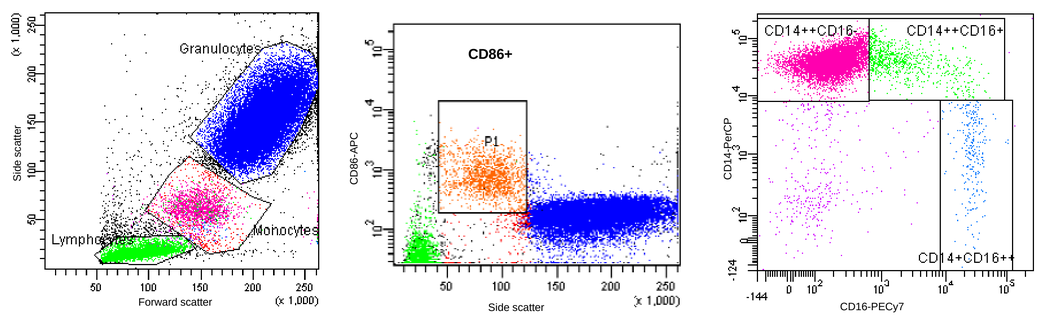


Incubation with uremic toxins

- Sodium citrate whole blood of healthy control
- Addition of mixture of:
  - sulfates:
    Indoxyl sulfate (44.5 mg/L), p-cresyl sulfate (43.0 mg/L), phenyl sulfate (13.5 mg/L)
  - glucuronides:
    Indoxyl glucuronide (3.9 mg/L), p-cresyl glucuronide (7.3 mg/L), phenyl glucuronide (1.6 mg/L)
- Incubation of 24h at 37°C

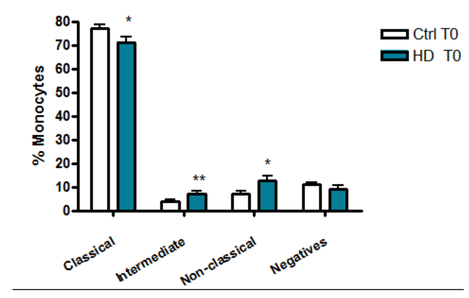
Flow cytometric analysis

Monocytes were identified based on the pan monocytic marker CD86 (APC labeled) and the 3 subpopulations were distinguished by their CD14 (PerCP) and CD16 (PECy7) expression



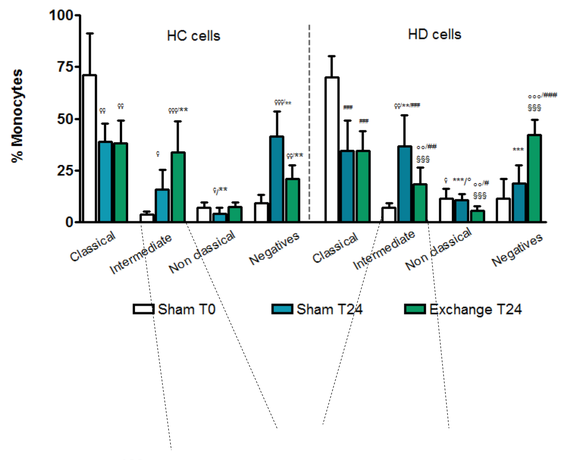
Results

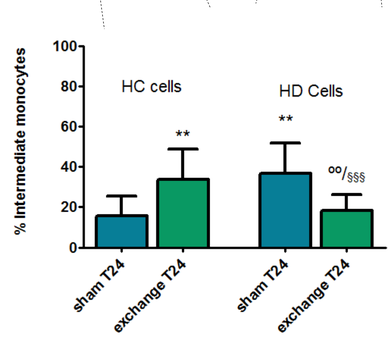
Baseline control vs HD



→ The **percentage** of **intermediate monocytes** and non-classical monocytes was significantly **increased** in HD patients at baseline .(n=8); *P<0.05; **P<0.01

Exchange experiment





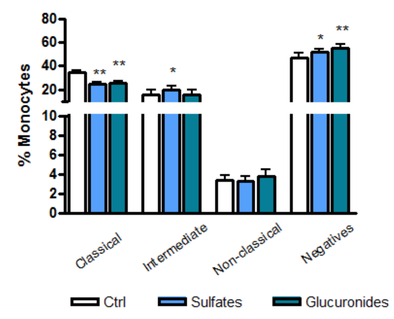
Statistics (n=8):
1 symbol: p < 0.05; 2 symbols: p < 0.005;
3 symbols: p < 0.001
Ç: vs HC sham T0
*: vs HC sham T24
°: vs HC exchange T24
#: vs HD sham T0
§: vs HD sham T24

→ **Incubation per se** induces a **shift** towards **intermediate monocytes**

→ **HD** patients have a **higher shift** towards the **intermediate monocytes** compared to healthy cells

→ **Exchange** of **healthy plasma with HD plasma** induces a significant increase **towards intermediates**, which was **reversible** as the exchange of HD plasma with healthy plasma results in a decreased shift in comparison to the HD sham condition and comparable to the healthy sham condition

Incubation of healthy blood with uremic toxins



→ Incubation of healthy blood with a **sulfate mixture** results in an **increase** of **intermediate monocytes** vs. control (n=8). * p < 0.05; ** p < 0,005

Conclusions

- **The increased percentage of intermediate monocytes observed in hemodialysis patients can, at least in part, be attributed to the presence of the uremic milieu → exchange of plasma: healthy cells show uremic differentiation pattern and vice versa**
- **The presence of protein bound uremic sulfates in uremic plasma contributes to the increased shift towards the pro-inflammatory intermediate monocytes**

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