DOES BETA-2-MICROGLOBULIN EXERT A VASCULAR DAMAGING EFFECT BY ACTIVATING LEUKOCYTES?

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1. Introduction

Chronic Kidney Disease (CKD)

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characterized by ↑ **oxidative stress** and chronic low-grade inflammation
=
non-traditional risk factor for cardiovascular disease in CKD

Beta-2-microglobulin (B2M) (MW 11.8 kDa)
=
1) traditional marker for middle molecules in CKD.

2) predictor **cardiovascular disease (CVD) and mortality** in the general population and CKD patients

Does B2M contribute actively to the inflammatory burden in CKD?
In this study we investigated whether B2M induces free radical production in leukocytes in vitro.

2. Methods

Human beta-2-microglobulin, isolated from urine (**hB2M**) (Merck-Calbiochem®); used at uraemic concentrations of 10 mg/l and 50 mg/l

Burst activation
Oxidative burst: (Phagoburst®, Orpegen, Heidelberg, Germany): at **baseline**, after stimulation with **fMLP** (formyl-Met-Leu-Phe), **E. coli** and **PMA** (phorbol-12-myristate-13-acetate)

Set-up: 100µl whole blood of **healthy** volunteers was incubated **in vitro** with test solutions (e.g. hB2M) and the production of reactive oxygen species (ROS) was measured by the oxidation of rhodamine (RDH-123) at baseline and after stimulation with fMLP, E.coli and PMA. This effect was compared to total uraemic milieu: whole blood of dialysis patients (pre-dialysis sample, **Ur**).
Free radical production was measured by ***flow cytometry*** (FACScan)

B2M-concentration quantification: ELISA (DRG Diagnostics)

Control of *LPS-contamination* by LAL-test, Kinetic QCL® (Lonza)

Purification of hB2M solution via a micro-dialyser membrane with membrane MW cut-off 10kDa (Slide-A-Lyzer®, Thermo Scientific, USA).
- 9 dialysate (DPBS) exchanges over 11h30.
- Dialysate samples: first exchange: **D1**(0-30min dialysis) and the last exchange: **D2** (9-11.5h)
- Sample: dialysed B2M (**dB2M**).

Statistical analysis: Kruskall Wallis, Friedman-Ranks, pairwise comparisons

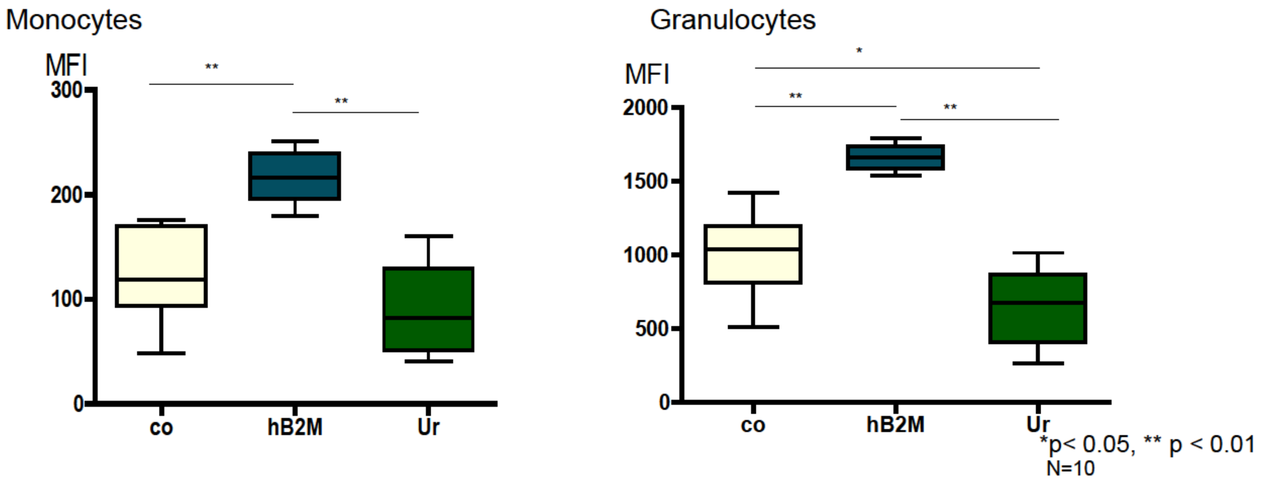
3. Results

Burst activity of hB2M

1) No effect on burst activation at baseline nor after stimulation with fMLP.
After stimulation with E.coli and PMA: strong stimulation of ROS in all leukocyte types

2) In comparison: effect stronger than oxidative burst induced by the entire uraemic milieu

e.g. after stimulation ***PMA***

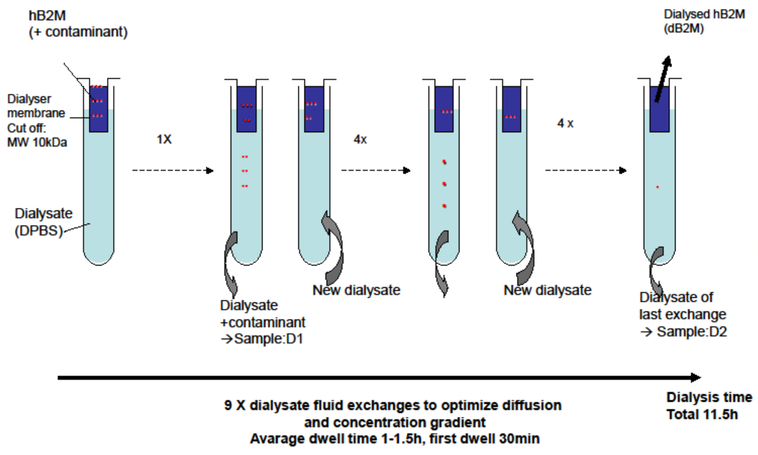


*p< 0.05, ** p < 0.01
N=10

Suspicion of contamination

1. ***LPS contamination?*** Determination of LPS in hB2M (50 mg/l): 1.5 EU/ml
→ No effect of LPS 1.5 EU/ml in the burst-test.

2. ***Other source?*** Dialysis of the hB2M-solution



Dialysed B2M (dB2M)
Concentration B2M = ok
LPS (50mg/l dB2M): 1.5 EU/ml

Dialysate samples
Concentration B2M = not detectable
LPS: < 0.025 EU/ml

Burst-test after purification: hB2M versus dB2M

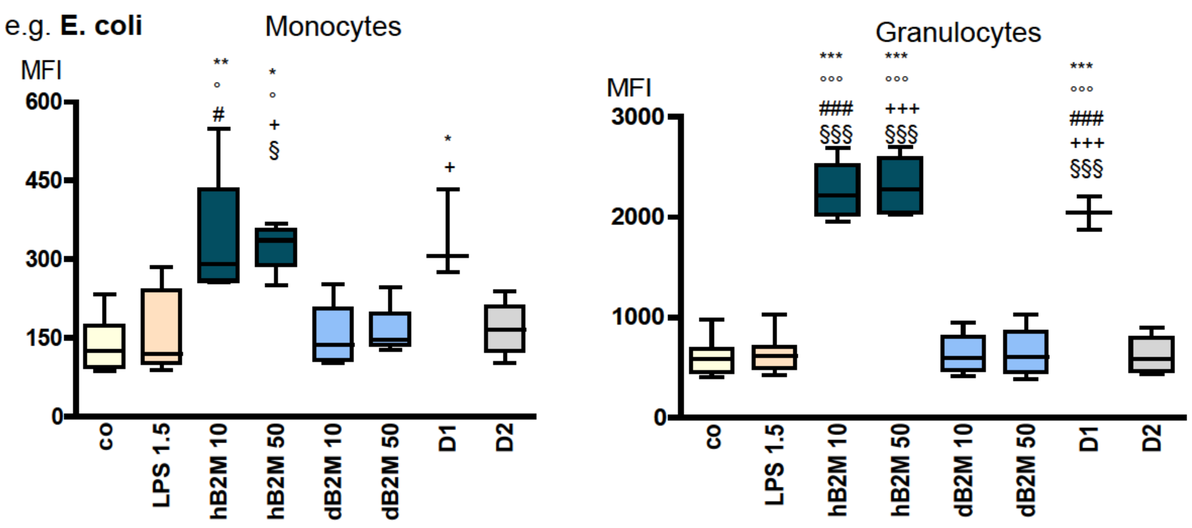
The results of the burst test demonstrated that the burst activation evoked by the hB2M solution was due to one or more contaminants with MW < 10 kDa.

1) The dialysate sample of the first dwell (**D1**) showed a **simular burst activation** in all types of leukocytes as **compared to the hB2M** solution (i.e. after stimulation with **E.coli and PMA**).

2) **dB2M** (purified B2M) could **not** induce free radical production in any condition.

3) **D2,** a sample of the last dialysate exchange, did **not** activate leukocytes in any condition, suggesting that all contaminants were cleared from the hB2M solution, at least to a concentration that did not induce burst activation anymore.

e.g. **E. coli**



*: p < 0.05,**: p < 0.01, ***: p < 0.001 vs.co, °: p < 0.05, °°: p < 0.01, °°°: p < 0.001 vs LPS, #: p < 0.05, ###: p < 0.001 vs dB2M10; +: p < 0.05, +++: p < 0.001 vs dB2M50; §: p < 0.05, §§§: p < 0.001 vs d2; co, LPS and hB2M: n=10, dB2M and D1,2: n=4

Conclusion

Beta-2-microglobulin, an emerging marker for cardiovascular outcome, does not contribute to micro-inflammation in chronic kidney disease by inducing leukocyte oxidative burst.
Other effects on leukocytes or other cell types involved in inflammation and cardiovascular disease still need to be determined.

Different sources of contamination can hamper the interpretation of biological experiments. This should be excluded, especially when unexpected results are observed.