Systemic, but not local, low-grade endotoxinemia increases plasma sCD163 independently of the cortisol response

Ermina Bach1, Niels Møller1, Jens Otto L Jørgensen1, Mads Buhl2 and Holger Jon Møller3

1Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark
2The Neonatal Intensive Care Unit, Aarhus University Hospital, Aarhus, Denmark
3Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark

Correspondence should be addressed to E Bach: bach.ermina@gmail.com

Abstract
Aims/hypothesis: The macrophage-specific glycoprotein sCD163 has emerged as a biomarker of low-grade inflammation in the metabolic syndrome and related disorders. High sCD163 levels are seen in acute sepsis as a result of direct lipopolysaccharide-mediated shedding of the protein from macrophage surfaces including Kupffer cells. The aim of this study was to investigate if low-grade endotoxinemia in human subjects results in increasing levels of sCD163 in a cortisol-dependent manner.

Methods: We studied eight male hypopituitary patients and eight age- and gender-matched healthy controls during intravenous low-dose LPS or placebo infusion administered continuously over 360 min. Furthermore, we studied eight healthy volunteers with bilateral femoral vein and artery catheters during a 360-min infusion with saline and low-dose LPS in each leg respectively.

Results: Systemic low-grade endotoxinemia resulted in a gradual increase in sCD163 from 1.65 ± 0.51 mg/L (placebo) to 1.92 ± 0.46 mg/L (LPS) at 220 min, P = 0.005 and from 1.66 ± 0.42 mg/L (placebo) to 2.19 ± 0.56 mg/L (LPS) at 340 min, P = 0.006. A very similar response was observed in hypopituitary patients: from 1.59 ± 0.53 mg/L (placebo) to 1.83 ± 0.45 mg/L (LPS) at 220 min, P = 0.021 and from 1.52 ± 0.53 mg/L (placebo) to 2.03 ± 0.44 mg/L (LPS) at 340 min, P < 0.001. As opposed to systemic treatment, continuous femoral artery infusion did not result in increased sCD163.

Conclusion: Systemic low-grade endotoxinemia resulted in increased sCD163 to levels seen in the metabolic syndrome in both controls and hypopituitary patients. This suggests a direct and cortisol-independent effect of LPS on the shedding of sCD163. We observed no effect of local endotoxinemia on levels of serum sCD163.

Introduction
CD163 is a cortisol-regulated monocyte and macrophage-specific surface glycoprotein and the extracellular portion of CD163 circulates in blood as a soluble protein (sCD163) (1).

sCD163 is highly elevated in acute infections including bacteremia and sepsis (2, 3, 4) and in inflammatory liver diseases (5, 6, 7, 8).

In vitro, lipopolysaccharide (LPS, endotoxin) induces ectodomain shedding of sCD163 by TLR-4 stimulation of the metalloproteinase TACE (9). LPS is a constituent of the outer membrane of the cell wall of gram-negative bacteria. LPS acts as a mediator of both acute and chronic inflammation (10, 11, 12, 13) and is an important mediator of gram-negative sepsis (14).
In vivo, high doses of LPS (from 2 ng/kg to 4 ng/kg) are used as a human model of the initial phase of sepsis (15), and in these settings, LPS administration increases plasma CD163 within minutes to the high levels seen in sepsis (9, 16).

Small, but significant, increases in sCD163 are seen in conditions of low-grade inflammation, such as obesity, insulin resistance and type 2 diabetes mellitus (T2D) (17, 18, 19, 20), but it is not known if these changes are directly linked to low-grade endotoxinemia or they are indirect, since LPS acts through a number of cytokines and also activates the hypothalamo-pituitary axis (HPA) and stimulates the release of stress hormones (e.g. cortisol) into the blood (21, 22, 23).

Since CD163 is strongly expressed in tissues such as liver, spleen and lungs, and only moderately expressed on monocytes, it has been suggested that the major fraction of the shedded sCD163 originates from the tissues, but this has not been thoroughly investigated in clinical experiments.

In two placebo-controlled human trials we, therefore, aimed (1) to compare the effects of LPS on levels of sCD163 in hypopituitary patients (HP) (in the absence of pituitary stress hormone responses) and control subjects (CTR) by infusing LPS systemically (intravenously) (‘the systemic study’) and (2) to investigate the local effect of LPS on levels of sCD163 in healthy volunteers by infusing LPS into the femoral artery with collection of blood from the femoral vein (‘the leg study’).

Methods

The systemic study

The study had a randomized placebo-controlled design with two different study days. We studied eight HP and eight matched control subjects (CTR) on two occasions separated by a minimum of 1 month: (1) during continuous isotonic saline infusion (placebo day) and (2) during continuous *Escherichia coli* endotoxin infusion of 0.06 ng/kg/h (LPS day) intravenously for 6 h, as previously described (24). Blood was sampled at 0, 60, 220 and 340 min for sCD163 analysis.

HP

As previously described (24), seven HP underwent operation for nonfunctioning pituitary adenoma and one patient for craniopharyngioma and all patients developed panhypopituitary insufficiency afterward. All patients received substitution therapy with hydrocortisone, thyroxine, testosterone and growth hormone. In addition, one patient received desmopresin therapy. Average treatment length was 9 years. All HP were healthy (beside their pituitary deficiencies) and did not receive other medications that may influence the results.

HP did not take growth hormone and desmopresin medication the last day before the study and hydrocortisone was discontinued on the study day. HP received i.v. hydrocortisone 80 mg (13 mg/h) continuously during the experiment (t = 0–360 min) to avoid acute cortisol deficiency. This high dosage was based on pilot experiments with lesser doses which induced signs of cortisol deficiency (low blood pressure, nausea, vomiting).

The leg study

As previously described (25), catheters were inserted into the femoral artery and vein of both legs in eight healthy male volunteers, and arterial catheters were used for infusion of either LPS (0.025 ng/kg/h) or placebo (saline), respectively in each leg, in a single-blinded randomized manner. Blood samples were taken from both venous catheters at 0, 60, 170 and 350 min for sCD163 analysis.

Both studies were approved by the Central Denmark Region Ethics Committee (M-2010-0076), in accordance with the Declaration of Helsinki. The study protocol was registered at www.clinicaltrials.gov (NCT01452958). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

ELISA for sCD163

We determined serum concentrations of sCD163 in duplicate samples that had been stored for up to 3 years at -80°C by use of an in-house sandwich ELISA on a BEP-2000 ELISA-analyzer (Dade Behring, Germany) (22). In each run, we co-analyzed control samples and serum standards with concentrations traceable to purified CD163. The inter-assay imprecision was <5% CV. The limit of detection was 6.25 μg/L. Soluble CD163 is robust to thawing and prolonged freezing (22).

Hyperinsulinemic–euglycemic clamp

As previously described (24), the study consisted of a 240-min basal period, followed by a 120-min hyperinsulinemic–euglycemic clamp period in the systemic study, and 180-min basal period and 180-min clamp period in the leg study (25).
Infusion rates of insulin during the clamp period (Insulin Actrapid; Novo-Nordisk) were 1.0 mU/kg/min i.v. Systemic plasma glucose was clamped at 5 mmol/L by a variable infusion of 20% glucose and arterial plasma glucose concentrations were measured at least every ten minutes (Beckman Instruments, Palo Alto, CA, USA).

Statistics
Data are presented as mean ± s.e.m. Statistical analysis was performed using paired t-tests comparing the effect of LPS vs placebo, unless otherwise stated. In the absence of normal distribution, P values were calculated by Wilcoxon signed-rank test. Normal distribution was assessed by inspection of QQ-plots.

Results
Effects of continuous systemic LPS infusion on serum concentrations of sCD163 (The systemic study)
Continuous intravenous LPS infusion in healthy controls resulted in an increase in sCD163 at 220 min (1.92 ± 0.46 mg/L LPS day vs 1.65 ± 0.51 mg/L placebo day, P = 0.005), and a further increase at 340 min (2.19 ± 0.56 mg/L LPS day vs 1.66 ± 0.42 mg/L placebo day, P = 0.006) (Fig. 1A). A similar increase was observed in HP at 220 min (1.83 ± 0.45 mg/L LPS day vs 1.59 ± 0.53 mg/L placebo day, P = 0.021) and at 340 min (2.03 ± 0.44 mg/L LPS day vs 1.52 ± 0.53 mg/L placebo day, P < 0.001) (Fig. 1B).

Effects of continuous local LPS infusion on serum concentrations of sCD163 (The leg study)
In contrast to the systemic LPS infusion, concentrations of sCD163 in the femoral vein were not significantly increased by local LPS infusion (Fig. 2).

Cortisol levels
As previously described (24), overall TW ANOVA for repeated measurements revealed a main LPS effect to increase cortisol levels (P < 0.001) in CTR and the difference was significant both during the first 240 min (‘basal period’): 88 ± 9 ng/mL (placebo) vs 138 ± 17 ng/mL (LPS), P = 0.017, and after 360 min (insulin stimulation – ‘clamp’) of the experiment: 94 ± 11 ng/mL (placebo) vs 211 ± 20 ng/mL, P = 0.002.

Cortisol levels in HP remained unaltered both during the first 240 min: 315 ± 35 ng/mL (placebo) vs 315 ± 31 ng/mL (LPS), P = 0.902, and after 360 min: 342 ± 37 ng/mL (placebo) vs 339 ± 35 ng/mL (LPS), P = 0.774. Main LPS effect to increase cortisol levels (TW ANOVA for repeated measurements) was also unaltered (P = 0.858). Cortisol levels were not measured in the leg study.

Glucose levels
The systemic study
As previously described (24), systemic plasma glucose was approximately at 5 mmol/L both in CTR and HP, and there was no difference between placebo and LPS days.
In CTR, glucose levels were 5.4 ± 0.1 mmol/L (placebo) vs 5.1 ± 0.1 mmol/L (LPS), \( P = 0.383 \), during the first 240 min, and after 360 min glucose levels were 4.9 ± 0.1 mmol/L (placebo) vs 4.9 ± 0.1 mmol/L (LPS), \( P = 0.872 \).

In HP glucose levels were 5.7 ± 0.3 mmol/L (placebo) vs 5.5 ± 0.1 mmol/L (LPS), \( P = 0.459 \), during the first 240 min, and after 360 min, glucose levels were 5.0 ± 0.1 mmol/L (placebo) vs 5.1 ± 0.1 mmol/L (LPS), \( P = 0.347 \).

The leg study
As previously described (25), overall TW ANOVA for repeated measurements revealed a main LPS effect to decrease glucose differences \( (P = 0.015) \) in the leg, although glucose arterio-venous differences during the clamp only reached borderline significance \( (P = 0.068) \) when tested separately.

Discussion
This study answers an important question in relation to the low-grade inflammation biomarker sCD163. We show in a randomized controlled design that low-dose systemic LPS directly and within hours increases sCD163 in plasma independently of a cortisol response. sCD163 is regarded as an important biomarker of low-grade inflammation which reflects insulin resistance and is related to increased diabetes risk. In our systemic study we mimicked low-grade inflammation by infusion of low-dose LPS and found a 25% consistent increase in sCD163, which is equivalent to the increase observed in obesity and diabetes. This increase did not depend on activation of the HPA axis, indicating a direct effect of LPS on monocytes and macrophages as seen in the in vitro and sepsis situations. In vitro, the cellular CD163 expression is upregulated by glucocorticoid after 12–24 h, and it is a possibility that our relatively short observation period failed to detect a more delayed cortisol effect. The HP patients in the study received 80 mg Solu-cortef over the 6 h – the same dosage during the placebo days and during the LPS days, however, with no effect on sCD163 on the placebo days. Taken together, our data demonstrate that the changes in sCD163 seen in low-grade inflammatory conditions may be directly mediated by low levels of LPS. We cannot, however, exclude that over time, regulatory mechanisms such as HPA activation may contribute to further increased CD163 expression and shedding.

The leg study shows no increase in venous sCD163 neither in the infused nor noninfused leg. Since there is no increase in sCD163 in the noninfused leg, this may indicate that only a minor fraction of the locally injected LPS reaches the systemic circulation. The lack of increase in the LPS-injected leg suggests that monocytes and tissue macrophages in the leg do not substantially contribute to the increase in sCD163 seen after systemic administration.

The strengths of our study include the randomized and placebo-controlled design and the inclusion of systemic vs local low-grade endotoxinemia. Moreover, we investigated the cortisol-independent effects of low-grade endotoxinemia on sCD163.

The study originates from a randomized study comparing saline infusion with LPS-induced inflammation’s effect on human metabolism during amino acids, fat and glucose tracers (isotopes) and insulin stimulation in the last part of the experiments. We do not, however, have any reason to believe that either tracers or insulin have affected sCD163 response.

Declaration of interest
Jens Otto L Jørgensen is a senior editor for Endocrine Connections. He was not involved in the review or editorial process for this paper, on which he is listed as an author. The other authors have nothing to declare.
References


7 Holland-Fischer P, Grønbaek H, Sandahl TD, Moestrup SK, Riggio O, Ridola L, Aagaard NK, Möller HJ & Vilstrup H. Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. *Gut* 2011 60 1389–1393. (https://doi.org/10.1136/gut.2010.234542)


11 Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *Journal of Endotoxin Research* 2004 10 201–222. (https://doi.org/10.1179/096805104225006129)


