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Basic Study

Anti-CD163-dexamethasone conjugate inhibits the acute phase response to lipopolysaccharide in rats

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Data sharing statement: Dataset is available from the corresponding author at karethom@rm.dk.

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Abstract

AIM: To study the effect of a new anti-CD163-dexamethasone conjugate targeting activated macrophages on the hepatic acute phase response in rats.

METHODS: Wistar rats were injected intravenously with either the CD163 targeted dexamethasone-conjugate (0.02 mg/kg) or free dexamethasone (0.02 or 1 mg/kg) 24 h prior to lipopolysaccharide (LPS) (2.5 mg/kg intraperitoneal). We measured plasma concentrations of...
INTRODUCTION

In conditions with macrophage proliferation and activation, CD163, a haemoglobin-haptoglobin scavenger receptor expressed exclusively on monocytes and macrophages, is up-regulated. Following toll-like receptor activation by inflammatory stimuli like lipopolysaccharide (LPS), receptor shedding to circulation as soluble CD163 (sCD163) is increased, and within hours upregulated on the cell surface. As an example, hepatic macrophages (Kupffer cells) are activated and sCD163 is increased in patients with liver cirrhosis who chronically experience some degree of endotoxaemia and acute phase response and this may be involved in the development of the serious cirrhosis complications.

We have recently constructed a conjugate of CD163 antibody and the potent corticosteroid dexamethasone (anti-CD163mAb-dexa) specifically targeting dexamethasone to activated macrophages. The conjugate reduces the LPS-stimulated cytokine release from activated macrophages in vitro and in vivo in rats and pigs. The effect is obtained with very low concentration of dexamethasone, thereby minimizing steroid-induced systemic effects. A fifty-fold higher concentration of non-conjugated dexamethasone is needed to obtain the same anti-inflammatory response.

Exposure to LPS is a standard method to induce an acute phase response with a large increase in pro-inflammatory cytokines and hepatic synthesis and release of acute phase proteins. While the conjugate reduces the LPS-mediated cytokine response in rats it remains unknown whether it also inhibits the hepatic acute phase protein synthesis response.

To approach this issue we measured the gene expression in liver tissue and serum concentrations of the prevailing acute phase protein α-2-macroglobulin (α-2-M) 24 h post-LPS exposure in rats. α-2-M is a hepatocyte-derived inhibitor of a wide range of proteinases that can be activated during inflammation. Further, we compared plasma concentrations of tumour necrosis factor-α (TNF-α) and interleukin 6 (IL-6) 2 h post-LPS exposure. Spleen weight served as an indicator of systemic steroid effects.

MATERIALS AND METHODS

Animals

The animal protocol was designed to minimize pain or discomfort to the animals. Female Wistar rats (body weight 190-210 g; Taconic M and B, Ejby, Denmark) were housed at 21 ± 2 °C with a 12-h artificial light cycle. Two or three animals were housed in each cage, with free access to tap water and standard food (Altromin, Lage, Germany) and acclimatized for one week. Food intake and body weight were registered at the beginning and at the end of the experimental procedures. The study was performed in accordance with local and national guidelines for animal welfare and approved by the national Animal Ethics Committee, protocol No. 2010/561-1918.

Design

Forty animals were allocated in 5 groups of 8: One...
control group receiving only vehicle (PBS pH 7.4) intravenously and four groups injected intravenously with either vehicle, anti-CD163mAb-dexa (0.02 mg/kg dexamethasone), high dose free dexamethasone (1 mg/kg) (Sigma-Aldrich, Brøndby, Denmark), or low dose free dexamethasone (0.02 mg/kg). The low (“therapeutic”) dose gives maximal steroid efficacy in other rat studies\textsuperscript{[14,15]} and the low dose was the same as in the anti-CD163mAb-dexa. After 24 h, 0.5 mL of saline (controls) or LPS dissolved in 0.5 mL saline (2.5 mg/kg) (from *Escherichia coli* 0111:B4 obtained from Sigma-Aldrich, Brøndby, Denmark; product No. L2630) was injected intraperitoneally. Two hours later and following anaesthesia with inhalation of isofluran 2%-3% (Forene\textsuperscript{a}, Abbott Laboratories, Gentofte, Denmark), a blood sample for determination of plasma TNF-\(\alpha\) and IL-6 was drawn from a retrobulbar venous plexus using heparinised micropipettes. After an overnight 12-h fast the animals were anesthetised with a subcutaneous injection of fentanyl/alfaniosone (Hypnorm\textsuperscript{a}, Jansen Pharmacia, Birkerød, Denmark) 0.5 mL/kg and midazolam (Dormicum\textsuperscript{a}, La Roche, Basel, Switzerland) 2.5 mg/kg. All blood was collected for blood analyses and approximately 200 mg of liver tissue was snap-frozen in liquid N\(\textsubscript{2}\), and stored at -80 \(\textdegree\)C. Finally, the spleen was weighed. In all animals we measured liver mRNA levels at termination of the study. Liver tissue mRNA levels of \(\alpha\)-2-M were determined by slot blot hybridization as previously described\textsuperscript{[16]}.

**Blood analyses**

The concentrations of \(\alpha\)-2-M in serum were evaluated by rat ELISA (Immunology Consultants Laboratory, Newberg, OR, United States). The plasma concentrations of TNF-\(\alpha\) and IL-6 were determined by immunooassay (R and D Systems, Minneapolis, MN, United States, both). Samples were analysed in duplicate and all assays had intra- and inter-assay coefficients of variance below 5\% and 10\%, respectively. Plasma concentrations of alanine aminotransferase and bilirubin were determined by standard clinical biochemical analytical methods.

**Statistical analysis**

Data were analysed using the Kruskal-Wallis One Way Analysis of Variance on Ranks; when significant, post-hoc tests were performed among groups by the Mann-Whitney rank sum test. Data are presented as the mean ± SEM. Differences were considered significant with \(P\)-values < 0.05. A statistical review of the study was performed by a biomedical statistician.

**RESULTS**

**Body and spleen weight**

LPS induced a body weight loss in all the intervention groups (\(P < 0.05\)) (Table 1) and there was no difference among these groups. The high dose dexamethasone dose decreased the spleen weight (\(P < 0.05\)), an effect not seen in any other group (Table 1).

**Acute phase protein liver mRNA and serum levels**

LPS increased the liver mRNA and serum levels of \(\alpha\)-2-M several fold in all groups (\(P < 0.01\)) (Figure 1). Anti-CD163mAb-dexa approximately halved the \(\alpha\)-2-M liver mRNA (\(P < 0.01\)) and serum response (\(P = 0.04\)) compared to low dose dexamethasone treated animals, while no free dexamethasone dose had any effect on liver mRNA or serum levels of \(\alpha\)-2-M compared to vehicle (Figure 1).

**TNF-\(\alpha\) and IL-6**

LPS markedly increased plasma TNF-\(\alpha\) and IL-6 in all groups (\(P < 0.001\)). There was a trend for reduced TNF-\(\alpha\) (\(P = 0.08\)) after anti-CD163mAb-dexa compared to vehicle and significantly so vs the low dose dexamethasone (\(P = 0.03\)). Also, the anti-CD163mAb-dexa decreased IL-6 compared to both dexamethasone doses (\(P < 0.05\)). None of the free dexamethasone doses had

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**Table 1 Weights, liver function tests, and cytokines.**

|                     | Controls | LPS       | Anti-CD163-dexa plus LPS | High dexa plus LPS | Low dexa plus LPS |
|---------------------|----------|-----------|--------------------------|--------------------|-------------------|
| Body weight (g)     | 199 ± 1  | 196 ± 2   | 207 ± 2\(^a\)           | 204 ± 3            | 206 ± 3\(^b\)    |
| Weight loss (g)     | 11 ± 5   | 14 ± 3    | 22 ± 2\(^a\)            | 23 ± 2\(^a\)       | 21 ± 1\(^a\)     |
| Spleen weight (mg)  | 465 ± 12 | 512 ± 31  | 492 ± 23                 | 421 ± 11\(^c\)     | 483 ± 23         |
| ALT (U/L)           | 42 ± 3   | 61 ± 16   | 57 ± 20                  | 48 ± 9             | 77 ± 31          |
| Bilirubin (mg/dL)   | 3.0 ± 0.0| 3.3 ± 0.3 | 3.1 ± 0.1                | 3.6 ± 0.4          | 4.0 ± 0.4        |
| TNF-\(\alpha\) (pg/mL) | 0 ± 0   | 26817 ± 9789\(^e\) | 204 ± 3                  | 204 ± 3            | 206 ± 3          |
| IL-6 (pg/mL)        | 0 ± 0    | 23075 ± 6758\(^e\) | 204 ± 3                  | 204 ± 3            | 206 ± 3          |

Body weight (g), body weight loss (g), spleen weight (mg), plasma alanine aminotransferase (U/L), and bilirubin (\(\mu\)mol/L) in controls (\(n = 8\)) and in animals injected with LPS 24 h after vehicle (\(n = 8\)), anti-CD163mAb-dexa (\(n = 8\)), high dose (\(n = 8\)) and low dose (\(n = 8\)) dexamethasone at termination of study. Plasma TNF-\(\alpha\) (pg/mL) and IL-6 (pg/mL) are measured 2 h after saline (controls) or LPS injection. \(^a\)\(P < 0.05\) vs controls; \(^b\)\(P < 0.05\) vs low dose free dexamethasone group; \(^c\)\(P < 0.05\) vs high dose free dexamethasone group; \(^d\)\(P < 0.05\) vs vehicle. ALT: Alanine aminotransferase; TNF-\(\alpha\): Tumor necrosis factor-\(\alpha\); IL-6: Interleukin-6; LPS: Lipopolysaccharide.
Anti-CD163-dexamethasone efficiently suppressed this response. The anti-inflammatory effects of glucocorticoids bind to the ubiquitous intracellular glucocorticoid steroid receptor present in most cell types they also exert serious systemic metabolic side effects. Thus dexamethasone causes the spleen to undergo a corticosteroid-induced weight reduction due to lymphocyte depletion\(^{[20]}\). Accordingly, the high dose dexamethasone in our study decreased the spleen weight as compared with the other groups reflecting systemic non-macrophages effects. In contrast, the conjugate did not affect spleen weight and was still found to exert a potent anti-inflammatory effect.

In our animal model, the conjugate was given as a pre-emptive dose prior to the induction of the acute phase response as we aimed at establishing a proof-of-concept position of the conjugate’s effects. We believe our findings support further studies on interference with on-going inflammation in relevant experimental models. Such studies are also essential for monitoring of long term effects of the conjugate.

In conclusion, the anti-CD163-dexa conjugate demonstrated potent effects in reducing the acute phase proteins without evident systemic side effects during an endotoxin-induced acute phase response in rats. The effect much exceeded that of a therapeutic dose of dexamethasone. Thus, the antibody conjugate may be a potential candidate in future anti-inflammatory macrophage-directed therapy, e.g., in liver diseases with Kupffer cells activation\(^{[7]}\). However, as glucocorticoids bind to the ubiquitous intracellular glucocorticoid steroid receptor present in most cell types they also exert serious systemic metabolic side effects. Thus dexamethasone causes the spleen to undergo a corticosteroid-induced weight reduction due to lymphocyte depletion\(^{[20]}\). Accordingly, the high dose dexamethasone in our study decreased the spleen weight as compared with the other groups reflecting systemic non-macrophages effects. In contrast, the conjugate did not affect spleen weight and was still found to exert a potent anti-inflammatory effect.

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**COMMENTS**

**Background**

In conditions with macrophage proliferation and activation, CD163, a scavenger
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receptor expressed exclusively on monocytes and macrophages, is upregulated. As an example, hepatic macrophages (Kupffer cells) are activated and CD163 is increased in patients with liver cirrhosis who chronically experience some degree of endotoxemia and acute phase response.

**Research frontiers**

The authors have recently constructed a conjugate of CD163 antibody and the potent corticosteroid dexamethasone (anti-CD163ImmAb-dexa) specifically targeting dexamethasone to activated macrophages.

**Innovations and breakthroughs**

The anti-CD163-dexa conjugate exerts an anti-inflammatory effect, which is obtained with very low concentration of dexamethasone, thereby minimizing steroid-induced systemic effects.

**Applications**

The antibody conjugate may be a potential candidate in future anti-inflammatory macrophage-directed therapy, e.g., in liver diseases with Kupffer cells activation.

**Peer-review**

This is an experimental report written by Thomsen et al, which indicates an efficacy of dexamethasone-conjugated anti-CD163 against lipopolysaccharide-induced acute inflammatory reaction. The well-designed study was carried out using firm methods.

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