DEVELOPMENT OF A CYTOMETRIC BEAD ARRAY SCREENING TOOL
Simultaneous detection of pro-inflammatory cytokines in plasma of lipopolysaccharide-challenged pigs

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Introduction

Lipopolysaccharide (LPS) has been widely used as a model of immune challenge in pigs as this compound induces the immediate synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-6. In research, multiplex assays currently are a very popular tool for simultaneous detection of biomarkers of infection and inflammation. Specific and sensitive Enzyme-Linked Immuno Sorbent Assays (ELISAs) are well-suited to perform single factor analysis, yet for multi-parameter analyses, this approach is time-consuming and expensive. Cytometric bead array (CBA) is a flexible, bead-based flow cytometric application for the simultaneous detection of various soluble proteins of interest. The aim of the present study was to develop and validate a CBA 3-plex assay for the major pro-inflammatory cytokines TNF-α, IL-1β and IL-6. The results were compared to commercial ELISA kits.

Materials and Methods

Experimental design

Four male pigs (Seghers Hybrid), with a mean (+ SD) body weight (BW) of 24.9 ± 3.17 kg were intravenously challenged with 15 µg ultrapure LPS/kg BW (Escherichia coli serotype O111:B4). ELISAs were purchased from R&D Systems.

CBA 3-plex assay for TNF-α, IL-1β and IL-6

An overview of the CBA assay is summarized and illustrated in Figure 1. More details of the protocol are described by Wyns et al. (2012).

Results

Table 1 reports the limits of detection (LODs), intra- and inter-assay variations (CVs) and dynamic ranges of the CBA 3-plex cytokine assay. Following an in vivo LPS challenge, similar plasma concentration-time profiles were observed for all cytokines with CBA and ELISA as shown in Figure 2.

Table 1. LOD, mean intra- and inter-assay CVs and dynamic ranges of each cytokine in the CBA 3-plex assay and commercially available ELISAs.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>LOD (ng/mL)</th>
<th>Intra-assay (CV%)</th>
<th>Inter-assay (CV%)</th>
<th>Dynamic range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.363</td>
<td>0.004</td>
<td>8.45</td>
<td>15.71</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.109</td>
<td>0.007</td>
<td>2.26</td>
<td>5.77</td>
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<tr>
<td>IL-6</td>
<td>0.005</td>
<td>0.002</td>
<td>2.33</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

CBA and ELISA show similar cytokine concentration-time profiles in plasma. Therefore, the optimised and validated CBA 3-plex cytokine protocol provides a fast, flexible and cost-effective screening tool for simultaneous measurement of the major porcine pro-inflammatory cytokines TNF-α, IL-1β and IL-6. This technique will be applied in future research to study the immunomodulatory properties of drugs in a porcine LPS inflammation model.