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 the 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).

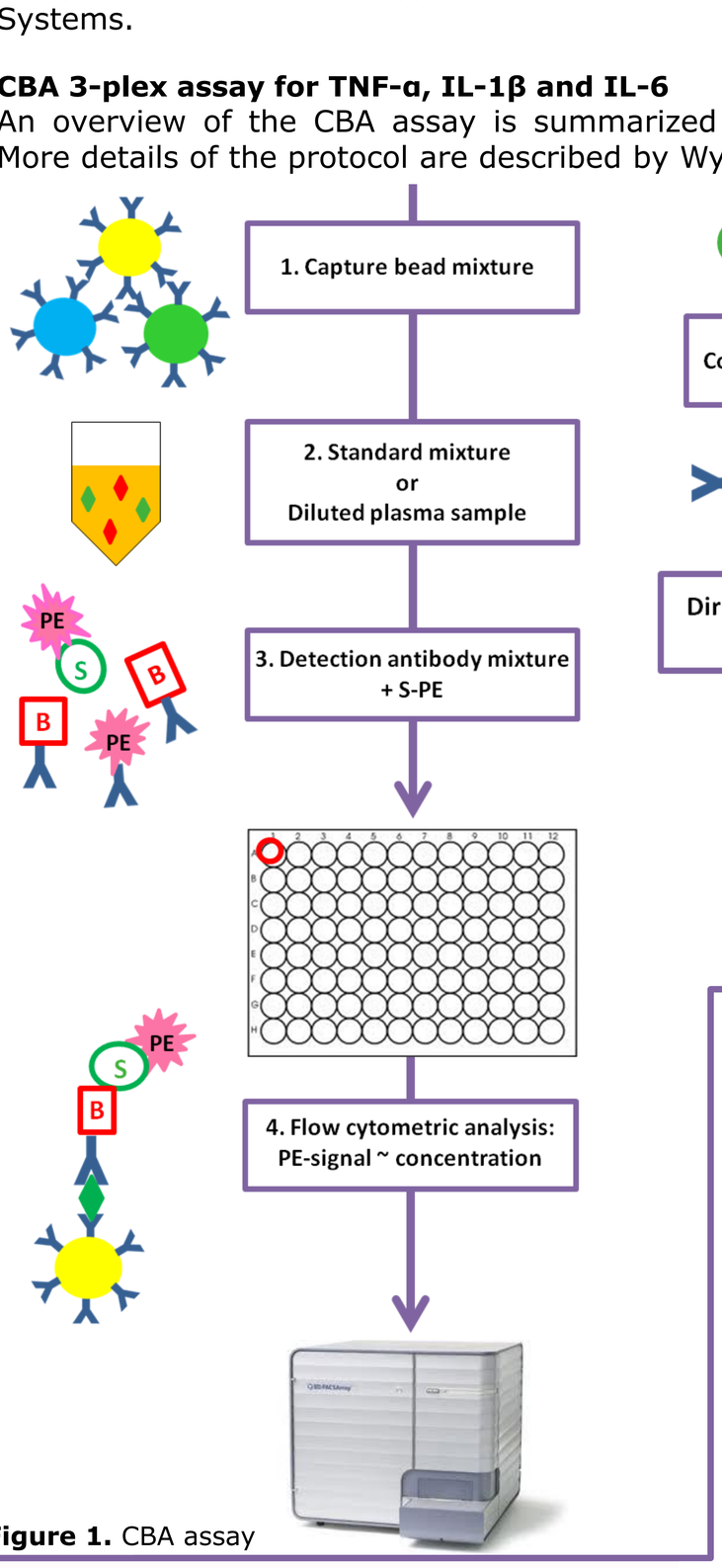

Figure 1. CBA assay

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.

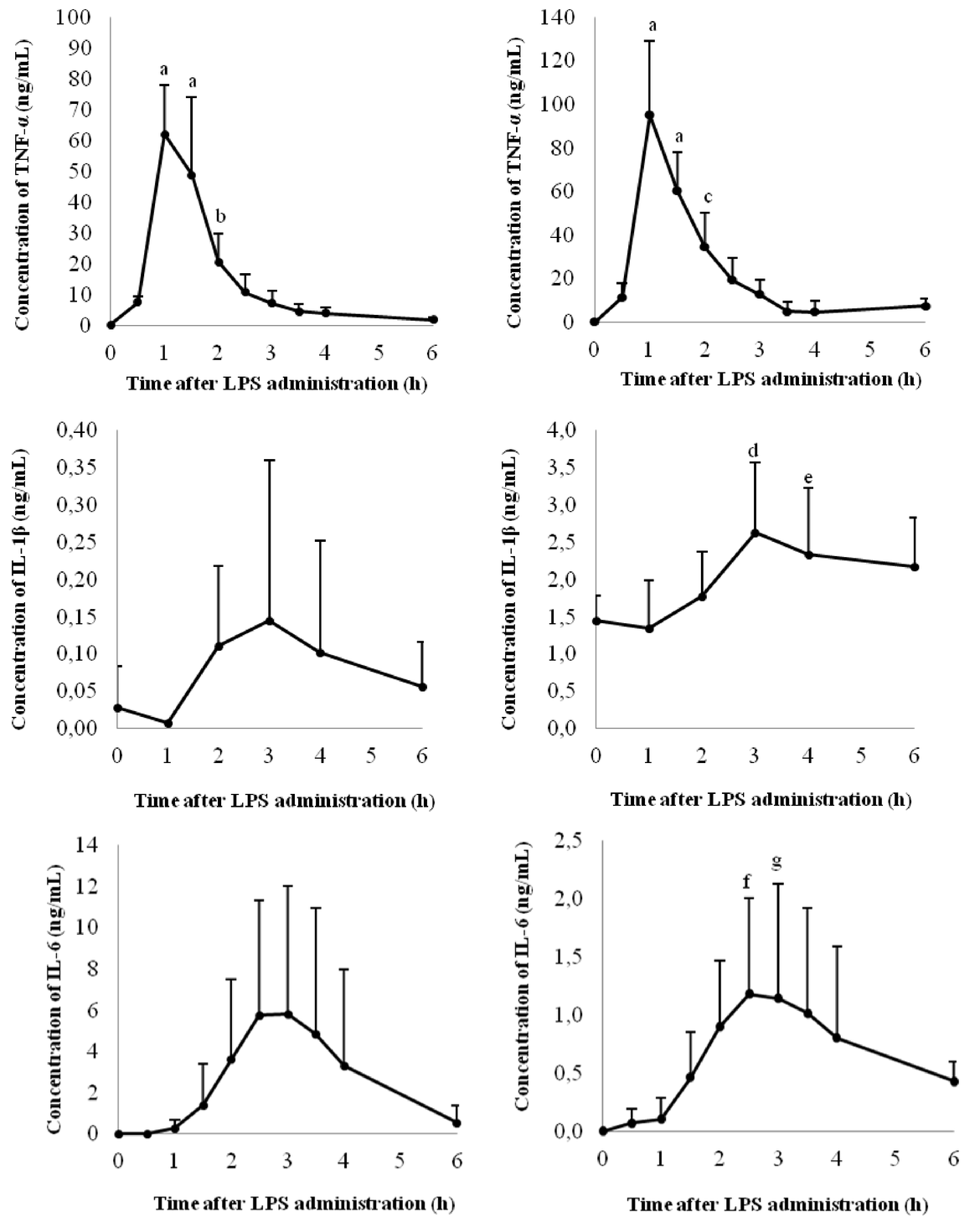

Figure 2. Mean (+ SD) plasma concentration-time curves of TNF-α, IL-1β and IL-6 obtained from LPS-challenged pigs (n = 4). Adjusted *p*-values are reported as a ($p < 0.0005$), b ($p = 0.033$), c ($p = 0.004$), d ($p = 0.005$), e ($p = 0.039$), f ($p = 0.019$) and g ($p = 0.026$). Concentrations were measured using both ELISA (left panel) and CBA (right panel). Note different scales on the y-axes for TNF-α, IL-1β and IL-6.

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.

Cytokine	LOD (ng/mL)		Intra-assay (CV%)		Inter-assay (CV%)		Dynamic range (ng/mL)	
	CBA	ELISA	CBA	ELISA	CBA	ELISA	CBA	ELISA
TNF-α	0.363	0.004	8.45	4.86	15.71	8.90	0.50 - 50	0.023 - 1.50
IL-1β	0.109	0.007	2.26	5.77	15.48	6.27	0.50 - 50	0.039 - 2.50
IL-6	0.005	0.002	2.33	4.00	11.41	5.97	0.50 - 50	0.010 - 1.20

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.

Wyns, H., Croubels, S., Demeyere, K., Watteyn, A., De Backer, P., Meyer, E., Development of a cytometric bead array screening tool for the simultaneous detection of pro-inflammatory cytokines in porcine plasma. Vet. Immunol. Immunopathol. (2012), http://dx.doi.org/10.1016/j.vetimm.2012.09.041 (in press)