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Letter to the Editor

Interference of canakinumab with commercial IL-1β ELISAs

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To the Editor:

Caspase-1/inflammasome pathways play a pivotal role in innate immune responses. Activated caspase-1 processes pro-IL-1β and pro-IL-18 into their activated secretory forms, thereby initiating a pro-inflammatory immune response. Dysregulated caspase-1 signaling is involved in the pathogenesis of several autoimmune/inflammatory conditions; therefore, analysis and drug based regulation of its signaling pathways are of high interest.

Recently, we optimized a low-volume human whole blood assay (WBA), facilitating the measurement of caspase-1 activation upon specific stimulation of the NLRP3, NLRC4 or AIM2 inflammasome [1]. The assay is based on secreted IL-1β, a surrogate of caspase-1 activation. In contrast to peripheral blood mononuclear cell based assays, soluble-plasma factors and cellular interactions are not lost within whole blood assay samples. Therefore, WBAs may mimic the patient's situation in a more physiological manner. Furthermore, as no cell isolation is needed, the assay requires comparably small sample volumes. However, WBAs can be limited by the presence of therapeutically used drugs or antibodies in patients' plasma. Analyzing blood samples from patients treated with canakinumab, we noted interferences between canakinumab and certain IL-1β ELISAs leading to false-negative IL-1β readouts. Canakinumab is a human IgG1/κ anti-IL-1β monoclonal antibody, which exclusively binds to human and marmoset IL-1β, thereby functionally neutralizing the biological activity of this cytokine [2–4]. Briefly, canakinumab binds to the n-terminus of mature IL-1β which builds the IL-1β epitope, with the amino acid Glu 64 being the key residue [5]. Canakinumab is approved for the treatment of autoinflammatory diseases cryopyrin-associated periodic syndrome (CAPS), Hyper-IgD syndrome (HIDS), familial Mediterranean fever (FMF) and TNF-receptor associated periodic syndrome (TRAPS), as well as for systemic juvenile idiopathic arthritis (SJIA) and gouty arthritis. Canakinumab displays pharmacokinetic properties typical for IgG1 antibodies with an elimination half-life of approximately 4 weeks. Typically, adults are treated with 150 mg canakinumab *s.c.* every eight weeks for CAPS, or every four weeks for HIDS, FMF and TRAPS. This results in canakinumab serum levels of 5–15 μg/ml [4].

As the WBA is based on plasma IL-1β as surrogate for inflammasome/caspase-1 activation, it is necessary to quantify total mature IL-1β even in the presence of canakinumab. In order to optimize the WBA for patients under canakinumab therapy, we screened various commercially available IL-1β ELISAs for their interference with canakinumab. Binding of canakinumab to IL-1β led to a dose-dependent decrease in IL-1β detection by all tested ELISAs (Fig. 1, A and B). However, IL-1β recovery varied greatly between the tested assays: As most assays displayed IL-1β recovery rates > 70% at canakinumab concentrations $\leq 10^{-3}$ μg/ml, only the R&D Quantikine HS and BD OptEIA ELISA detected > 50% of the IL-1β input at canakinumab concentrations of 1–10 μg/ml which are typically found in patients. Of note, the Biolegend LegendPlex and R&D Quantikine ELISAs showed a minimum recovery of IL-1β at canakinumab concentrations of 0.1–1.0 μg/ml, again gaining better recovery at higher canakinumab concentrations. Previously, authors used fixed correction factors to adjust ELISA based IL-1β readouts in canakinumab treated patients [4]. However, all ELISAs tested in this study showed a dose-dependent inhibition of IL-1β recovery. Hence, applying fixed correction factors may introduce bias. If applicable, normalization of experimental samples to control samples within one patient may correct the bias induced by canakinumab.

Taken together, of five tested ELISAs, the Biolegend LegendPlex, R&D Quantikine HS and BD OptEIA were able to determine IL-1β in the presence of 1–100 μg/ml canakinumab. However, the measured values are not directly comparable to samples without or with differing canakinumab concentrations.

Conflicts of interest

The authors declare no conflict of interest in relation to the presented work.

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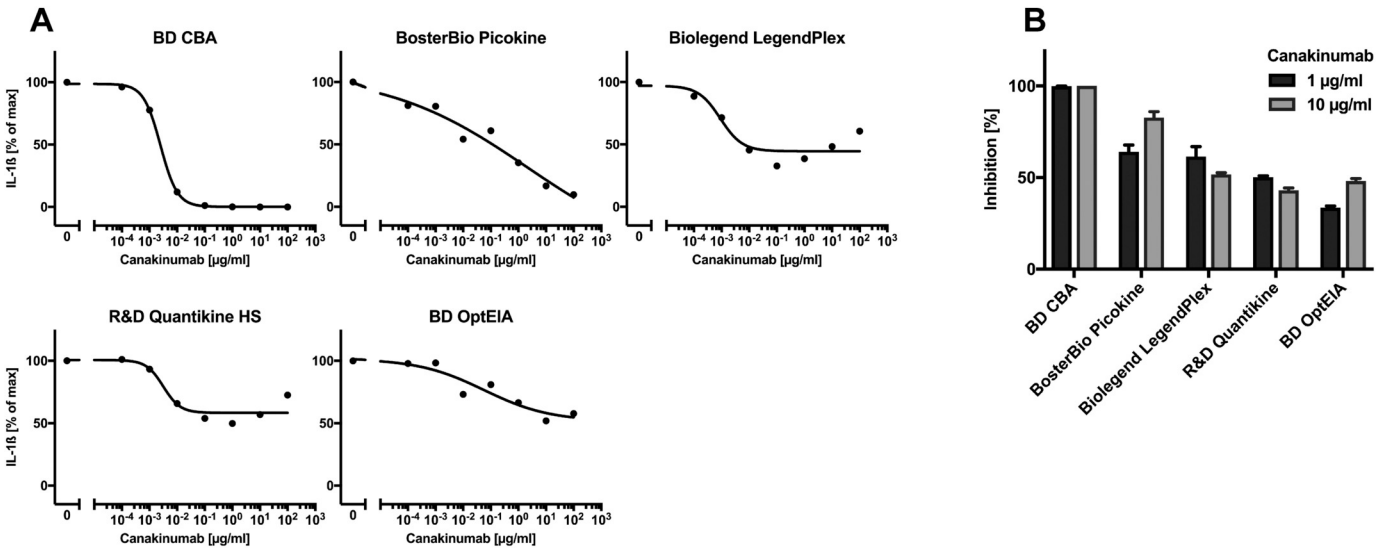

Fig. 1. Canakinumab inhibits IL-1β detection.
Recombinant human IL-1β standard (500 pg/ml; 558457, BD Biosciences) was incubated with increasing concentrations of canakinumab (Ilaris®, Novartis Pharma) in RPMI1640 with 10% FCS (4 h, room temperature). Subsequently, IL-1β concentrations were analyzed with plate-, or bead-based IL-1β ELISAs (BD OptEIA, 557966; BosterBio PicoKine, EK0392; R&D Systems Quantikine HS, HSLB00D; BD CBA Flex-Set, 558279 and 558264; BioLegend LegendPlex, 740669) according to the manufacturer's protocol. A, Data are presented as percentage of maximum IL-1β detection at 0 pg/ml canakinumab. B, Data are presented as percentage of assay inhibition for 1 and 10 µg/ml canakinumab.

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