SELECTIVE INHIBITION OF THE CANNABINOID RECEPTOR CB1 FOR THE TREATMENT OF INFLAMMATION AND FIBROSIS

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Abstract

Cannabinoid receptor CB1 inhibition has been proposed as a promising therapeutic approach to treat metabolic diseases, whereas CB2 receptor activation has been proposed as a promising therapeutic approach for the treatment of inflammation and fibrosis. Our current lead drug candidate lenabasum is a selective CB2 agonist that shows anti-inflammatory and anti-fibrotic effects in animal models and human subjects. Lenabasum is currently in phase 3 clinical trials for treatment of systemic sclerosis and dermatomyositis and phase 2 for treatment of cystic fibrosis and systemic lupus. However, it remained to be determined whether CB1 inhibition can ameliorate inflammation and fibrosis, an effect that can be useful for treating metabolic diseases that are accompanied by inflammation and fibrosis, such as non-alcoholic steatohepatitis (NASH). To answer this question, we selected a compound from a library of rationally designed peripherally restricted CB1 inverse agonists. This compound (CRB-4001) displayed over 1000-fold CB1/CB2 binding selectivity and was shown to improve metabolic parameters in diet induced diabetic mice. We first confirmed that CRB-4001 is highly potent in both CB1-mediated cAMP and βarrestin2 assays (low and sub-nanomolar, respectively). Then, we evaluated the effect of CRB-4001 on the inflammatory response of human PBMCs. We found that CRB-4001 reduced the LPS-induced pro-inflammatory cytokines MCP-1, IL-1β, TNFα, IL-6, IL-12, IL-17 and IL- 31 in a dose dependent manner (low μM potency) without affecting IL-8. In the BioMAP® phenotypic profiling platform that utilizes twelve human primary cell-based disease models, CRB-4001 reduced the levels of several tissue remodeling biomarkers in fibroblasts, including PAI-1, TIMP-1, type I and type III collagens and αSMA. These changes were accompanied by decreases in pro-inflammatory cytokines as well as inhibitory effects on cell proliferation in multiple BioMAP systems. The anti-inflammatory and anti-fibrotic activities together with a low brain/plasma exposure ratio indicated that CRB-4001 is a promising candidate for NASH.

Methods

Receptor binding.
Membrane homogenates from CHO cells that overexpress either CB1 or CB2 were incubated for 120 min at 37°C with 0.5 nM [^{3}H]CP 55940 or 0.8 nM [^{3}H]WIN 55212-2 in a buffer that contained 0.3% or 0.1% BSA, respectively, in the absence or presence of CRB-4001. The results are expressed as a percent inhibition of the control radioligand specific binding.

CB-1 mediated cAMP and βarrestin2 recruitment were evaluated using the Hit Hunter Enzyme Fragment Complementation (EFC) assays with β-galactosidase (β-Gal) as the functional reporter. For Gi-mediated cAMP in an inverse agonist mode, cells were pre-incubated with CRB-4001 in the presence of EC20 forskolin. For Gi-mediated βarrestin2 inhibition (antagonist mode), cells were pre-incubated with CRB-4001 followed by agonist (CP55,940) challenge at the EC80 concentration.

LPS-induced cytokine release from human PBMC. PBMCs isolated from a healthy volunteer with increasing concentrations of CRB-4001 for 2 hours. 1 μg/ml dexamethasone (DEX) served as a positive control. LPS was added at a final concentration of 0.1 μg/ml and further incubated for 24 hours. At the end of the incubation, the supernatants were collected and the levels of a panel of secreted cytokines were measured by a Human Magnetic Luminex® assay (R&D). Cytotoxicity was evaluated using the CytoTox-one Homogenous Membrane Integrity Assay Kit (Promega) that measures the level of LDH release.

BioMAP® phenotypic profiling platform was used to evaluate the effects of CRB-4001 on multiple biomarkers of immune response, tissue remodeling, proliferation and fibrosis. Human primary cells in this platform were incubated with the indicated concentrations of CRB-4001, stimulated and assayed as described[2]. Readouts at each concentration were divided by the average of vehicle control samples to generate a ratio that was $\log_{10}$ transformed.

CRB-4001 is a potent CB1 selective inverse agonist

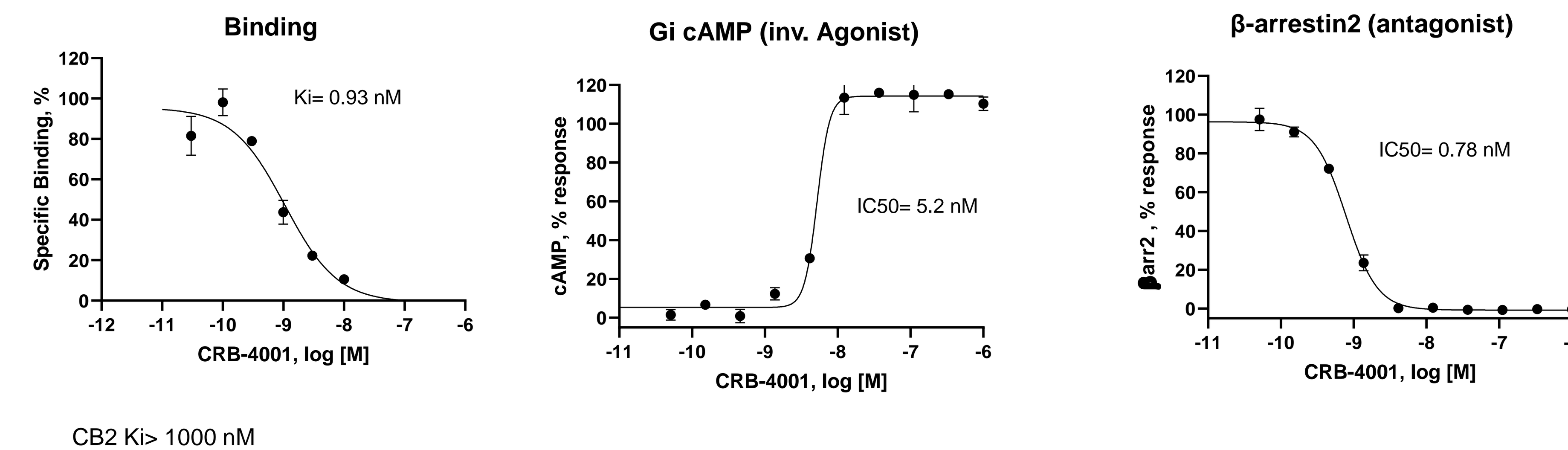


Figure 1. CRB-4001 receptor pharmacology was evaluated by competitive binding to CB1 and CB2 receptors (left), CB1-dependent cAMP assay in an inverse agonist mode (middle) and by a β-arrestin 2 recruitment to CB1 assay (right). Binding was evaluated in membrane homogenates from CHO cells that overexpress either CB1 or CB2. Bound ^{3}[H] CP55,940 or ^{3}[H] WIN 55212-2, to CB1 or CB2 receptors respectively, were displaced by incubation with the indicated concentrations of CRB-4001. cAMP levels and β-arrestin 2 recruitment to CB1 were measured using the DiscoverX Enzyme Fragment Complementation (EFC) with β-galactosidase (β-Gal) as the functional reporter.

CRB-4001 reduces inflammatory cytokines in human PBMC

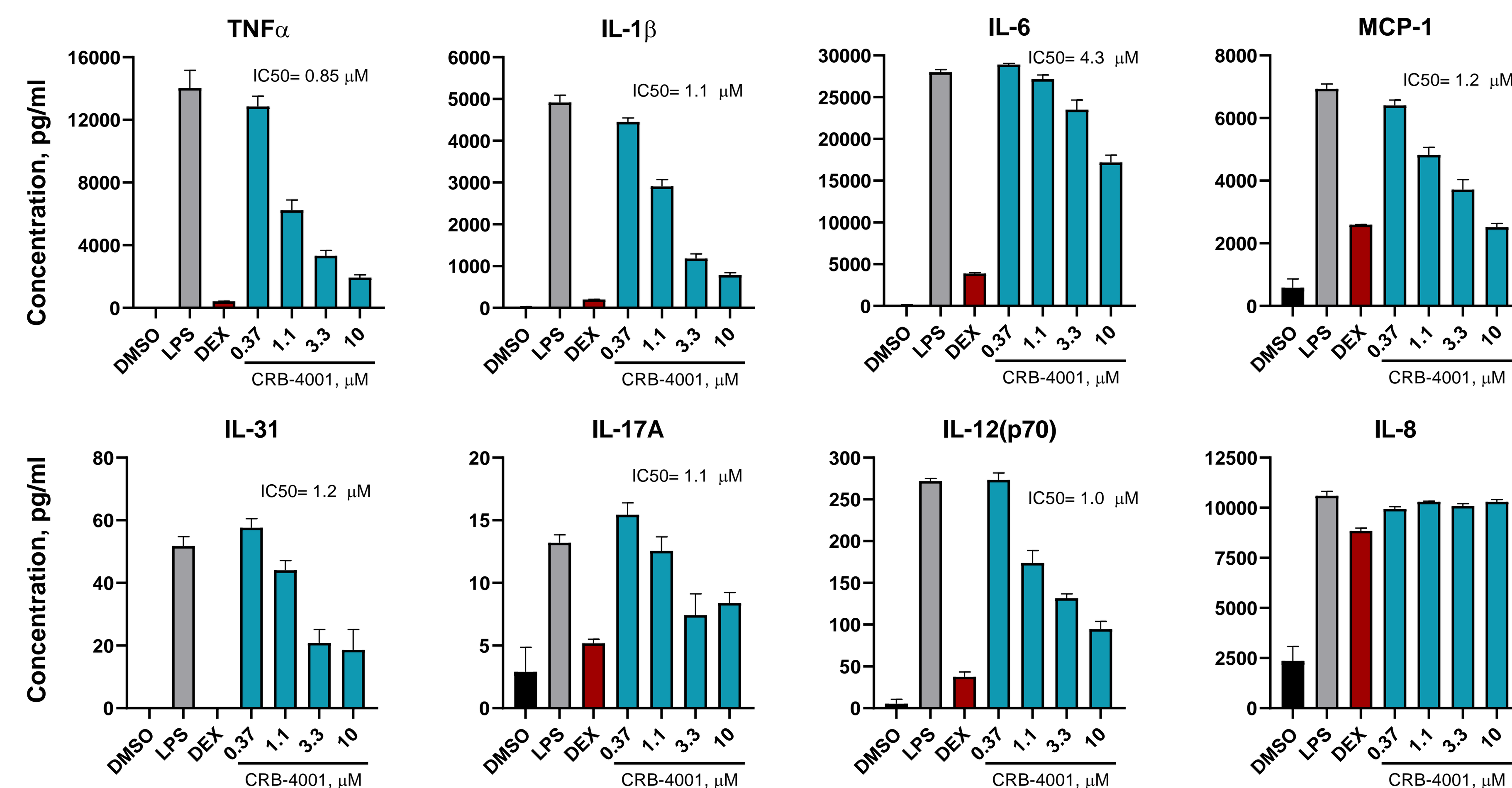
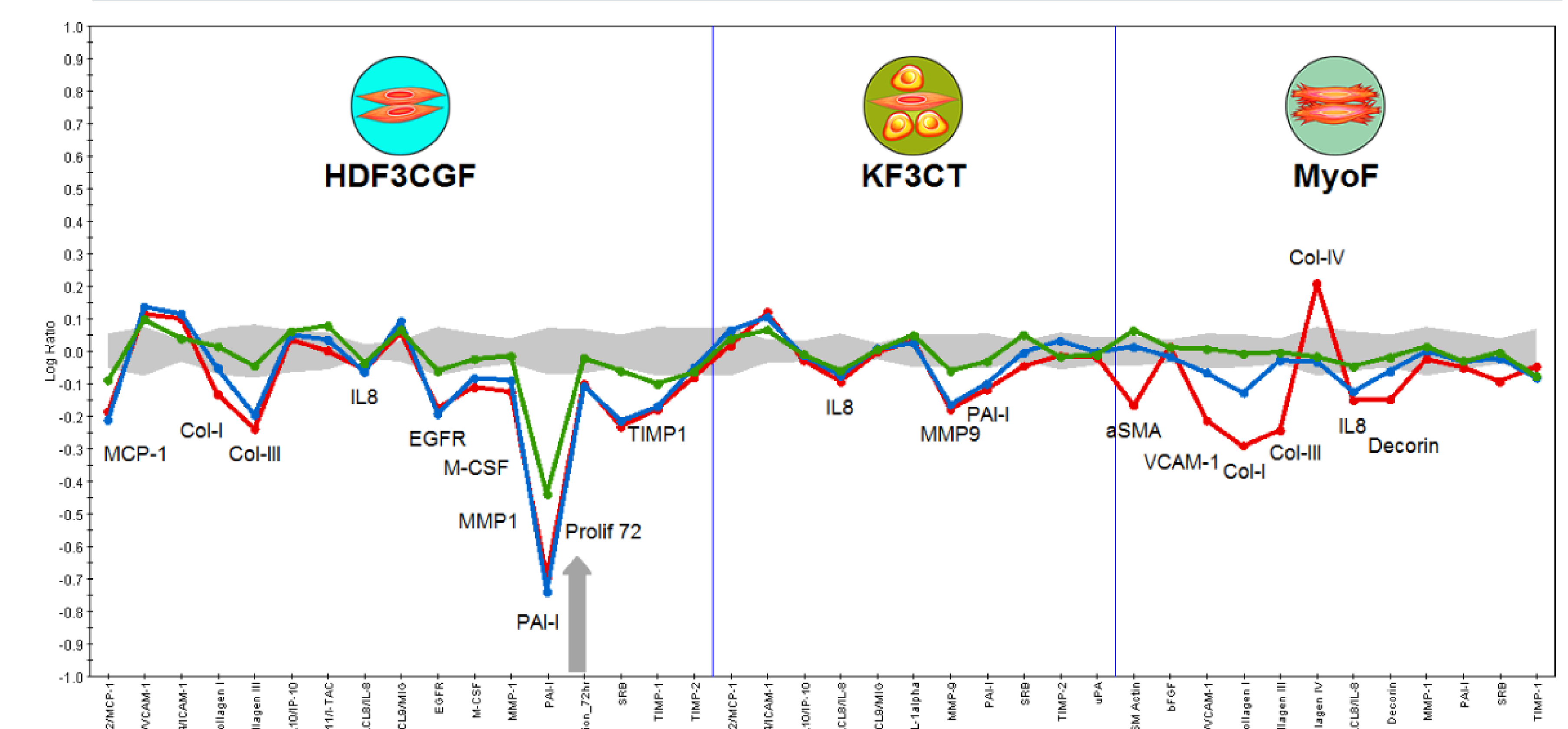


Figure 2. CRB-4001 was assayed for its effect on inflammatory cytokine release from lipopolysaccharides (LPS)-induced human Peripheral Blood Mononuclear Cells (PBMC). PBMC were isolated from a blood sample of a healthy volunteer, counted and cultured in triplicates. Cells were incubated with the indicated concentration of CRB-4001 for 2 hours, treated with 100 ng/ml LPS and incubated for 24 hours. 1 μg/ml dexamethasone (DEX) served as a positive control. At the end of the incubation, the supernatants were collected and the levels of a panel of secreted cytokines were measured by a Human Magnetic Luminex assay. Cytotoxicity levels in all treatment groups was similar to DMSO control, assessed by the levels of Lactate Dehydrogenase (LDH) release

Conclusions

- CRB-4001 suppressed the induction of pro-inflammatory cytokines in human primary cells
- CRB-4001 affected multiple aspects of the immune response, by suppressing the activations of monocytes, T cells and B cells
- CRB-4001 blocked the proliferation of stimulated B cells, T cells and dermal fibroblasts
- CRB-4001 suppressed multiple extra cellular matrix (ECM) and pro-fibrotic biomarkers, including PAI-1, TIMP-1, type I and type III collagens and αSMA
- CRB-4001 is a promising drug candidate for metabolic diseases that are accompanied by inflammation and fibrosis, such as NASH

CRB-4001 reduces inflammation and fibrosis in the BioMAP platform



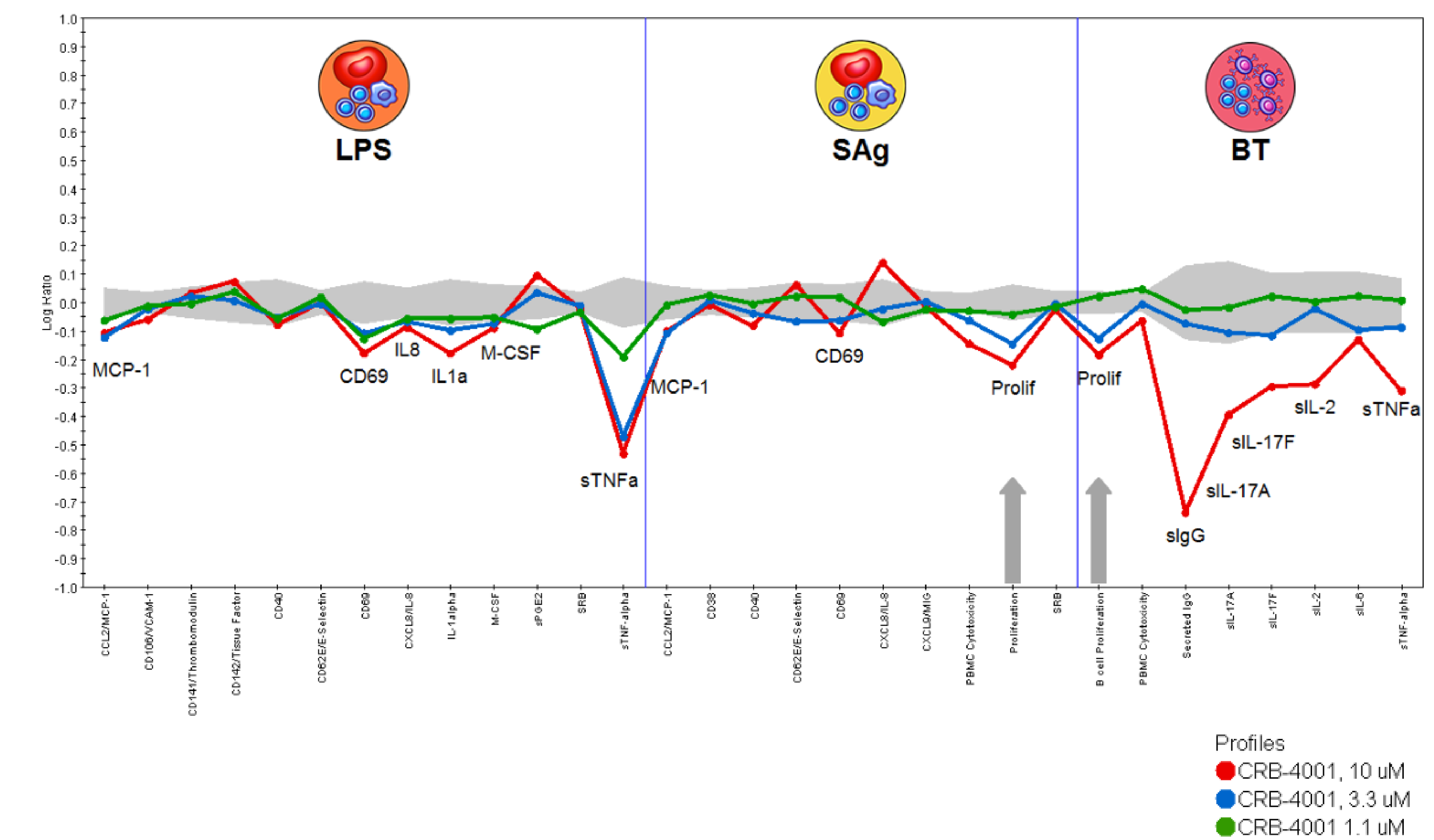


Figure 3. Human primary dermal fibroblasts (HDF3CGF) were cultured alone and co-cultured with human primary keratinocytes (KF3CT). Human primary lung fibroblasts (MyoF) were cultured alone. Human primary endothelial cells (LPS and SAg systems) or CD19+ B cells (BT system) were co-cultured with human primary PBMC. All cells were pooled from multiple healthy donors. Systems were stimulated and assayed as previously reported[2]. Data for biomarker measurements of compound-treated samples at each concentration were divided by the average of vehicle control samples to generate a ratio that was $\log_{10}$ transformed. Grey shaded area represents readouts of historical vehicle controls. Anti-proliferative are denoted by grey arrows.

References

1.Tam et al, *Cell Metabolism* 2012; 16(2)
2.Shah et al, *Cell Chemical Biology* 2017; 24(7)

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