Minor cannabinoids have pleiotropic effects on human brain microvascular endothelial activation and barrier function

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Abstract

Background

Disruption of the blood–brain barrier is a key driver of neuroinflammation during systemic inflammatory states. While minor phytocannabinoids, including cannabinol (CBN), cannabigerol (CBG), and cannabidiol (CBD), are recognized for their potential vascular and immunomodulatory actions, their direct impact on human brain endothelial barrier function under cytokine stress has not been systematically evaluated.

Methods

Primary human brain microvascular endothelial cells (HBMEC) from two donors were treated with TNF-α or IL-1β (0.1 ng/mL) in the presence or absence of CBN, CBG, or CBD (0.01–10 μM). Barrier function was assessed between 0–10 h using transendothelial electrical resistance (TEER). The structural integrity of tight and adherens junctions was assessed using immunofluorescence for Zonula Occludens-1 (ZO-1), Vascular endothelial (VE)-cadherin, and F-actin. IL-6 and IL-8 levels were measured using ELISA.

Results

TNF-α and IL-1β each induced a rapid and sustained reduction in HBMEC TEER, consistent with barrier dysfunction and junctional disruption, as assessed by immunofluorescence microscopy of ZO-1, VE-cadherin, and F-actin. Across both donors, CBN attenuated TNF-α–induced reductions in TEER and suppressed TNF-α–stimulated IL-6 and IL-8 release ($p < 0.05$), and partially restored VE-cadherin organization towards baseline, but not ZO-1 or F-actin. In contrast, CBN showed minimal or donor-variable effect under IL-1β, with no reproducible effect on TEER. CBD had little or no effect in the TNF-α model, and at higher concentrations, exacerbated IL-1β-driven permeability of HBMEC from one donor ($p < 0.05$). CBD selectively reduced IL-1β–induced IL-8 secretion with limited impact on IL-6. CBG at 10 μM augmented reductions in TEER induced by IL-1β or TNF-α ($p < 0.05$), and variably affected IL-1β or TNF-α-induced IL-6 and IL-8 release.

Conclusions

CBN partially reverses TNF-α-induced HBMEC barrier dysfunction and IL-6 and IL-8 production. In contrast, CBD shows a narrow, cytokine-specific anti-inflammatory signal, with partial suppression of IL-1β–induced IL-8 without barrier protection. Finally, CBG potentiates IL-1β and TNF-α-induced permeability, and is pro-inflammatory at higher concentrations. Our study identifies CBN as the most promising of these minor cannabinoids for TNF-α–driven inflammatory states, with associated endothelial activation and dysfunction, such as sepsis. These findings underscore that cannabinoid

actions on brain endothelial cells are both ligand- and cytokine-dependent and highlight the need to match cannabinoid choice and dosing to the prevailing inflammatory milieu.

Background

Endothelial barrier dysfunction is a critical pathological feature of systemic inflammation and plays a pivotal role in the development of organ injury during critical illness[1]. Within the central nervous system, disruption of the blood–brain barrier (BBB) caused by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), contributes to cerebral edema, neuroinflammation, and secondary neuronal damage[2]. Human brain microvascular endothelial cells (HBMEC), which form the structural and functional basis of the BBB, are particularly vulnerable to inflammatory insults, making them an important cellular model for studying barrier integrity under pathological conditions[3].

Cannabinoids have recently garnered growing interest due to their immunomodulatory and barrier-protective properties [4, 5]. While Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most studied phytocannabinoids, increasing attention has been paid to minor cannabinoids such as cannabinol (CBN) and cannabigerol (CBG)[6]. Preclinical studies suggest that CBD and CBG may confer vascular or anti-inflammatory benefits; however, their effects on endothelial barrier function remain inconsistent and context-dependent[7, 8]. In contrast, evidence regarding the vascular actions of CBN is sparse, despite its emerging popularity for symptom management, including pain, anxiety, and sleep disturbances[9].

Given the critical role of BBB integrity in regulating neuroinflammation and preventing secondary neuronal injury, a systematic comparison of the effects of minor cannabinoids on endothelial barrier function is warranted[10]. Although previous research has primarily focused on the neuroprotective and anti-inflammatory properties of THC and CBD, considerably less is known about the vascular actions of CBN and CBG[11]. Importantly, different minor cannabinoids may act through distinct receptor pathways and intracellular signaling mechanisms, suggesting that they could differentially modulate endothelial responses to inflammatory stimuli[12].

Human brain microvascular endothelial cells (HBMEC) are widely used as a representative model of the cerebral endothelium to evaluate blood-brain barrier (BBB) function in vitro[3]. Transendothelial electrical resistance (TEER) is a well-established quantitative method for assessing dynamic changes in barrier integrity and permeability[13]. Structural evaluation of endothelial junctions is commonly performed by immunofluorescence staining for VE-cadherin, the major adhesion molecule regulating endothelial cell–cell contacts and vascular barrier stability [14]. In addition, the quantification of cytokines such as IL-6 and IL-8 in cell culture supernatants provides insight into inflammatory activation and BBB dysfunction[15]. Together, these complementary approaches provide a robust framework for studying cytokine-induced endothelial barrier disruption and the protective effects of cannabinoids.

Growing evidence suggests that cannabinoids exert context-dependent effects on vascular and endothelial biology. Recent work highlights the emerging interest in phytocannabinoids as modulators of

endothelial dysfunction and inflammatory vascular injury, particularly in conditions such as COVID-19 where endothelial activation plays a central role[16]. In parallel, advances in cannabinoid biosynthesis have emphasized that many minor cannabinoids remain poorly characterized at both the structural and functional levels, underscoring a critical gap in understanding how less abundant phytocannabinoids, such as CBN and CBG, interact with endothelial pathways[17]. Beyond mechanistic insights, purified CBD has demonstrated improvements in human vascular endothelial function, including reductions in arterial stiffness and an increase in carotid artery diameter, suggesting that select phytocannabinoids may exert clinically relevant vascular effects[18]. Together, these findings underscore both the therapeutic potential and the knowledge gaps surrounding cannabinoids in endothelial regulation, highlighting the need for systematic evaluation of minor cannabinoids under defined inflammatory conditions.

We hypothesized that minor cannabinoids would differentially affect the inflammatory activation of HBMEC. We therefore investigated how the minor cannabinoids CBN, CBD, and CBG modulate the barrier function of HBMEC exposed to the pro-inflammatory cytokines TNF-α and IL-1β, two key mediators of systemic inflammation and sepsis-associated encephalopathy. Barrier integrity was assessed using TEER measurements and immunofluorescence microscopy, and endothelial inflammation was assessed by quantifying IL-6 and IL-8 in culture supernatants. Our goal was to identify minor cannabinoids that are potentially capable of improving endothelial barrier function under inflammatory stress, thereby providing a rationale for subsequent mechanistic and translational studies.

Methods

Cell Culture. Primary human brain microvascular endothelial cells (HBMEC) from two independent donors were purchased from AnaBios (Kirkland, WA, USA). Cells were cultured in EGM®-2 MV microvascular endothelial cell growth medium-2 bullet kit (Lonza, Walkersville, MD) and used at passage 5. HBMEC from donor A (Lot# 376.01.05.01.2F) and donor B (Lot# 376.07.05.01.2F) were used for all experiments.

Cytokine and Cannabinoid Treatments. Cells were stimulated with TNF-α (0.1 ng/mL; R&D Systems, Cat# 10291-TA) or IL-1β (0.1 ng/mL; R&D Systems, Cat# 201-LB). CBN, CBD, and CBG were purchased (Cayman Chemical, Ann Arbor, MI, USA) as 1 mg/mL stock solutions in methanol (MeOH), Cannabinoids were applied at final concentrations of 0.01–10 μM, and the final concentrations of vehicles (MeOH) were maintained at ≤ 0.1% in all conditions.

Transendothelial electrical resistance (TEER) Measurement. TEER was measured using Electric cell-substrate impedance sensing (ECIS) as described[19]. Primary HBMEC were seeded onto array plate and allowed to grow to confluence, reaching a stable plateau of barrier resistance. Upon reaching this stable plateau phase, treatments were applied, and this moment was defined as time 0. TEER was continuously monitored using an ECIS Zθ system (Applied BioPhysics, NY, USA) at a frequency of 4000 Hz. TEER values were normalized to levels at time 0. Barrier dysfunction was quantified as the reduction in area under the curve (ΔAUC) over a 10-hour experimental period (0–10 h) post-treatment.

Immunofluorescence Microscopy. Barrier integrity was also assessed by immunofluorescence staining for the tight junction protein ZO-1, adherence junction protein VE-cadherin, and actin cytoskeleton (F-actin) at t = 10 h. The primary antibody against ZO-1 (Cat. #339194, diluted 1:50) was obtained from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA). The primary antibody against VE-cadherin (Cat. #37085, diluted 1:50) was obtained from Cayman Chemical (Ann Arbor, MI, USA). F-actin staining was performed using a fluorescently labeled phalloidin conjugate (Cat. #ZK1129; Vector Laboratories, Newark, NJ, USA). Images were acquired using a KEYENCE BZ-X810 fluorescence microscope with identical exposure settings.

ELISA. IL-6 and IL-8 were quantified in HBMEC supernatants collected at the 10-hour time point using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems/Bio-Techne; Minneapolis, MN, USA) as per the manufacturer's instructions.

Statistics. All data are expressed as mean ±standard error of the mean (SEM) from a minimum of three independent experiments (n = 5–8 replicates per condition). Statistical analyses were performed using the GraphPad Prism software. Differences between groups were determined using either one-way or two-way analysis of variance (ANOVA), as appropriate for the experimental design. For all ANOVAs, post-hoc multiple comparisons were conducted using Tukey’s honest significant difference test. Statistical significance was set at $P < 0.05$.

Results

Effect of TNF-α and IL-1β on HBMEC barrier function.

Treatment of HBMEC from Donor A and Donor B with TNF-α or IL-1β induced a marked loss of barrier integrity compared with medium alone, reflected by a robust reduction in TEER over time and a negative ΔAUC (Fig. 1–3; Supplementary Fig. S1-S3). Given this reproducible cytokine-induced barrier dysfunction baseline, we next evaluated whether CBG, CBD, or CBN modulates cytokine-driven changes in TEER. Donor A data are presented in the main figures, and the corresponding Donor B results are shown in the Supplementary figures.

CBN effects on TNF-α– and IL-1β–induced barrier dysfunction across donors.

Across two HBMEC donors, CBN showed a stimulus-dependent and donor-modulated barrier-protective profile (Fig. 1; Supplementary Fig S1).

TNF-α-induced permeability

In Donor A, CBN at 0.1 and 10 μM significantly reduced the ΔAUC towards medium values, whereas 0.01 and 1 μM had no significant effect (Fig. 1A). CBN also partially reversed TNF-α-induced permeability of HBMEC from Donor B but only at CBN concentrations of 10 μM, but not 0.01–1 μM (Supplementary Fig S1A). Thus, CBN reproducibly counteracted TNF-α–mediated barrier dysfunction in both donors, albeit with a higher effective concentration in Donor B.

IL-1β-induced permeability

In contrast to the results for TNF-α, CBN again had only weak and donor-dependent effects on IL-1β-induced permeability of HBMEC from Donors A and B (Fig. 1B). In Donor A cells, CBN at 0.01–1 μM did not significantly change ΔAUC compared with IL-1β alone, whereas 10 μM CBN modestly reduced IL-1β-induced changes toward medium alone (Fig. 1B). In Donor B cells, CBN alone had no effect compared with medium, and 1 μM CBN slightly augmented IL-1β–induced HBMEC permeability, as reflected by a further decrease in ΔAUC relative to IL-1β alone (Supplementary Fig S1B).

CBD has limited effects on HBMEC barrier function and augments IL-1β-induced permeability at higher concentrations.

Across HBMEC from both donors, CBD displayed minimal barrier-modifying activity, with its effects varying by both inflammatory stimulus and donor (Fig. 2; Supplementary Fig. 2). Overall, CBD did not confer meaningful stabilization of barrier function, and at higher concentrations, it exacerbated cytokine-induced barrier dysfunction.

TNF-α-induced permeability

In Donor A, CBD at all tested concentrations (0.01–10 μM) did not affect either the TEER trajectory or ΔAUC of TNF- α-activated HBMEC, suggesting that it has neither protective nor detrimental effects (Fig. 2A). Donor B exhibited a similar lack of response at low and intermediate doses (0.01–1 μM); however, 10 μM CBD produced a small but significant increase in the ΔAUC, suggesting a modest barrier-preserving effect restricted to this high concentration and this donor (Supplementary Fig. 2A).

IL-1β-induced permeability: In contrast, under IL-1β stimulation (0.1 ng/mL), high concentrations of CBD augmented the permeability of HBMEC from both Donors. In Donor A HBMEC, CBD concentrations of 1 μM and 10 μM, but not 0.01 or 0.1 μM, significantly decreased ΔAUC (Fig. 2B). Donor B HBMEC exhibited a similar pattern: CBD concentrations of 10 μM, but not 0.01, 0.1 or 1 μM, significantly reduced ΔAUC, demonstrating additional exacerbation of barrier dysfunction relative to IL-1β alone (Supplementary Fig. 2B). These results indicate that CBD can exacerbate IL-1β–induced barrier dysfunction.

CBG Augments Permeability Induced by TNF-α or IL-1β.

Across both HBMEC donors, lower concentrations of CBG did not affect TEER at concentrations from 0.01-1 μM, but markedly augmented both TNF-α and IL-1β-induced reductions in TEER at a concentration of 10 μM (Fig. 3; Supplementary Fig. S3).

TNF-α-induced permeability

Under TNF-α stimulation, HBMEC from both donors exhibited the expected decline in normalized resistance with markedly negative ΔAUC. In Donor A, CBG at 0.01–1 μM did not alter either the TEER

trajectory or ΔAUC compared with TNF-α alone (Fig. 3A). In contrast, 10 μM CBG further decreased ΔAUC, indicating this high concentration exacerbates TNF-α–induced barrier dysfunction.

A similar pattern was observed in response of HBMEC from Donor B (Supplementary Fig S3A). Low and intermediate concentrations (0.01–1 μM) again had no detectable impact on barrier function relative to TNF-α alone. However, 10 μM CBG significantly exacerbated the reduction in TEER, producing the most negative ΔAUC among all conditions. These data confirm that at high concentrations, CBG reproducibly augments TNF-α-induced HBMEC permeability.

IL-1β-induced permeability

Under IL-1β stimulation, CBG showed a dose-dependent tendency to worsen permeability (Fig. 3B; Supplementary Fig. S3B). In HBMEC from Donor A, treatment with CBG at 0.01–1 μM did not affect IL-1β–induced reductions in TEER as compared with IL-1β alone. Notably, 10 μM CBG strongly augmented the IL-1β-induced decrease in ΔAUC, suggesting the CBG further disrupts IL-1β-induced endothelial dysfunction. The responses of Donor B cells paralleled those of Donor A cells. Low to intermediate concentrations (0.01–1 μM) did not affect TEER or ΔAUC, whereas 10 μM CBG significantly aggravated ΔAUC, representing the strongest IL-1β–induced permeability increase among all conditions tested.

Immunofluorescence microscopy suggests CBN partially ameliorates TNF-α-induced disruption of the endothelial barrier.

Immunofluorescence microscopy of Donor A HBMEC demonstrated that TNF-α (0.1 ng/mL) induced marked endothelial junctional disruption (Fig. 4A). Compared with medium control, ZO-1 staining changed from a continuous belt-like pattern to discontinuous, fragmented borders, VE-cadherin became irregular and weakened at cell–cell contacts, and F-actin redistributed from a cortical rim to prominent central stress fibers, consistent with cytoskeletal contraction and increased permeability.

TNF-α Treatment

CBN alone (10 μM) did not alter junctional morphology as compared with medium. When co-applied with TNF-α, CBN partially restored VE-cadherin organization towards baseline, with more continuous junctional labeling and fewer obvious gaps. However, ZO-1 continuity and F-actin stress fiber formation were only minimally affected and remained broadly similar to TNF-α alone.

IL-1β-Treatment

Compared with medium, treatment with IL-1β (0.1 ng/mL) induced major changes in the endothelial barrier, including discontinuity of VE-cadherin and ZO-1 and robust formation of actin stress fibers. CBN 10 μM had no effect on basal junctional structure, and it did not ameliorate barrier dysfunction, restore junctional continuity or reduce stress fiber formation induced by IL-1β.

TNF-α and IL-1β robustly induce IL-6 and IL-8 secretion in HBMEC across donors.

To validate the inflammatory responsiveness of our model, we first quantified IL-6 and IL-8 in supernatants from HBMEC (Donor A and Donor B) exposed to TNF-α or IL-1β. Relative to medium controls, both cytokines significantly increased IL-6 and IL-8 secretion in each donor line (Fig. 5–6; Supplementary Fig. S4-S5), confirming a robust and reproducible cytokine-driven inflammatory activation baseline for subsequent evaluation of cannabinoid modulation.

Low concentrations of CBN attenuate TNF-α–driven IL-6 and IL-8 production across donors, highlighting consistent low-dose anti-inflammatory actions.

To assess whether CBN-induced changes in endothelial barrier function were paralleled by alterations in inflammatory activity, IL-6 and IL-8 concentrations were measured in HBMEC supernatants after 10 h of exposure to TNF-α alone, CBN alone, and TNF-α plus CBN.

IL-6 secretion

For Donor A, low concentrations of CBN (0.01–0.1 μM) significantly reduced TNF-α–induced IL-6, with the strongest suppression at 0.1 μM (Fig. 5). At higher concentrations (1 and 10 μM), IL-6 levels remained lower than TNF-α alone, but the magnitude of inhibition was weaker. Similarly for Donor B, CBN at 0.01–0.1 μM significantly decreased IL-6 (Supplementary Fig S4). Notably, at the highest concentration (10 μM), IL-6 levels rebounded markedly and were significantly higher than those induced by TNF-α alone, indicating a loss of suppression and a paradoxical enhancement of cytokine release.

IL-8 secretion

For Donor A, all concentrations of CBN (0.01–10 μM) significantly reduced TNF-α-induced IL-8 production (Fig. 5). For Donor B, CBN concentrations of 0.01–1 μM significantly reduced TNF-α-induced IL-8 production, but the highest concentration of CBN (10 μM) had no effect on TNF-α-induced IL-8 production (Supplementary Fig S4).

CBN Differentially Modulates IL-1β–Induced IL-6 and IL-8 Production in a Donor-Dependent Manner

IL-6 secretion

In Donor A, CBN concentrations of 0.01–10 μM did not significantly change IL-1β–induced IL-6 (Fig. 6). In contrast, in Donor B, all CBN concentrations (0.01–10 μM) further increased IL-1β–stimulated IL-6, with statistically significant elevations across doses and a consistent upward shift relative to IL-1β alone (Supplementary Fig. S5).

IL-8 secretion

In Donor A, CBN at 0.01–10 μM did not significantly alter IL-8 levels, which remained similar to those in the IL-1β group (Fig. 6). By contrast, in Donor B, all CBN concentrations (0.01–10 μM) augmented IL-1β-induced IL-8 secretion (Supplementary Fig. S5). Thus, there is donor to donor variability in the effects of CBN on IL-1β-induced IL-8 production.

Figure 6. Cannabinoids modulate IL-1β–induced IL-6 and IL-8 secretion by Donor A HBMEC.

HBMEC were stimulated with IL-1β (0.1 ng/mL) for 10 h in the presence of CBN, CBG, or CBD (0.01–10 μM). CBN did not alter IL-6 or IL-8 at any dose. CBG increased IL-6 at 0.01–1 μM and reduced IL-8 at 10 μM. CBD increased IL-6 at 10 μM and modestly reduced IL-8 at 0.01–0.1 μM. Data are mean ± SEM; one-way ANOVA. *$p < 0.05$, **$p < 0.01$, **$p < 0.001$, ns = not significant.

CBD Concentration-Dependently Enhances TNF-α–Induced IL-6 and IL-8 Production by HBMEC.

IL-6 Secretion

For Donor A HBMEC, CBD concentrations of 0.01 and 1 μM did not affect TNF-α-induced IL-6 secretion (Fig. 5). In contrast, CBD at 10 μM increased IL-6 secretion, resulting in the largest cytokine response among all CBD-treated groups. For Donor B HBMEC, CBD at 0.01, 0.1, 1 and 10 μM concentration-dependently enhanced TNF-α–induced IL-6 production (Supplementary Fig S4).

IL-8 Secretion

For Donor A HBMEC, CBG (0.01–10 μM) did not affect TNF-α-induced IL-8 production (Fig. 5). For Donor B, all concentrations of CBG (0.01–10 μM) augmented TNF-α-induced IL-8 production (Supplementary Fig S4). These data indicate a pro-inflammatory effect of CBG.

CBD Has Limited Effects on IL-1β–Induced Production of IL-6 but Selectively Suppresses IL-8 Production, Particularly in Donor B.

IL-6 secretion

In Donor A, CBD at 0.01–1 μM did not significantly modify IL-1β–induced IL-6, whereas 10 μM CBD further elevated IL-6, yielding the highest IL-6 levels among CBD-treated groups and a significant increase relative to IL-1β alone (Fig. 6). In Donor B, CBD concentrations from 0.01–10 μM did not significantly alter IL-6 production (Supplementary Fig. S5).

IL-8 secretion: In Donor A, CBD at 0.1 and 1 μM, but not 0.01 μM and 10 μM produced a modest reduction in IL-1β-induced IL-8 production (Fig. 6). In Donor B, CBD showed a more consistent inhibitory profile: all concentrations of CBD (0.01–10 μM) significantly reduced IL-1β–induced IL-8 secretion, with similar degrees of suppression across the dose range (Supplementary Fig. S5).

CBG Potentiates TNF-α-Driven IL-6 and IL-8 Production by HBMEC.

IL-6 secretion

CBG induced a concentration-dependent augmentation of cytokine production. For Donor A, CBG concentrations of 0.01, 0.1 and 10 μM, but not 1 μM increased TNF-α-induced IL-6 production (Fig. 5). In contrast, for Donor B, treatment with CBG at concentrations of 0.01, 0.1, 1 or 10 μM did not significantly affect TNF-α-induced IL-6 secretion (Supplementary Fig S4). Thus, CBG does not exert an inhibitory effect on IL-6 production in Donor B.

IL-8 secretion

For Donor A HBMEC, CBG at concentrations of 0.01–10 μM did not alter TNF-α–induced IL-8 secretion (Fig. 5). For Donor B HBMEC, CBG (0.01–10 μM) augmented TNF-α-induced IL-8 production, with high IL-8 secretion by cells treated with CBG concentrations of 1 and 10 μM (Supplementary Fig S4). Thus, CBG robustly augments TNF-α–induced IL-8 release by Donor B HBMEC.

CBG Induces Modest, Concentration-Dependent Changes in IL-1β–Induced IL-6 and IL-8 Production with Donor-Specific Patterns.

IL-6 secretion

In Donor A, CBG concentrations of 0.01, 0.1 and 1 μM, but not 10 μM augmented IL-6 production by IL-1β-activated HBMEC (Fig. 6). In Donor B HBMEC, CBG concentrations from 0.01-10 μM had no significant effect on IL-1β–induced IL-6 (Supplementary Fig. S5).

IL-8 secretion

For Donor A, CBG concentrations of 0.01–1 μM had no effect on IL-1β–induced IL-8 production, whereas 10 μM CBG significantly reduced IL-8 secretion (Fig. 6). In Donor B, CBG affected IL-8 differently (Supplementary Fig. S5). CBG at 0.01 μM and 1 μM significantly reduced IL-1β–induced IL-8 production, whereas 0.1 μM and 10 μM had no significant effect.

Discussion

This study reveals a coherent pattern of ligand- and cytokine-specific modulation of the human brain microvascular endothelium. Across two HBMEC donors, CBN reproducibly and robustly attenuated TNF-α–induced barrier dysfunction, restoring TEER, limiting permeability, and suppressing TNF-α–driven IL-6 and IL-8 release. In contrast, CBN's actions on IL-1β-activated HBMEC were weak and donor-variable, without consistent barrier rescue and even mild worsening barrier dysfunction in Donor B. In contrast, CBD failed to protect barrier integrity under either cytokine; high doses tended to aggravate IL-1β-induced permeability, yet CBD selectively reduced IL-1β–induced IL-8 (especially in Donor B) with little effect on IL-6. These findings point to a narrow, chemokine-focused anti-inflammatory signal that is uncoupled from protection of the endothelial barrier structural. CBG showed the least favorable profile of the three cannabinoids, providing no barrier benefit under TNF-α or IL-1β and consistently exacerbating

permeability at 10 μM, in several settings. Additionally, CBG treatment led to increased cytokine release. Collectively, our findings identify CBN as the most promising TNF-α–targeted barrier-protective candidate among the three minor cannabinoids, highlight CBD's limited but specific modulation of the IL-1β–IL-8 axis, and underscore that cannabinoid effects on the BBB are highly dependent on cytokine milieu, concentration of the cannabinoid, and donor background.

Although CBN clearly attenuated TNF-α–induced barrier dysfunction at the functional level, its effects on individual junctional and cytoskeletal markers were not uniform. In Donor A, CBN partially improved VE-cadherin organization after TNF-α exposure, whereas ZO-1 and F-actin remained largely indistinguishable from TNF-α alone. One possibility is that CBN preferentially stabilizes adherens junctions while exerting only modest influence on tight junction scaffolding and actin remodeling, so that changes in ZO-1 and stress fibers are too subtle to be captured by qualitative immunofluorescence at the 10 h time point. Alternatively, TEER may integrate small, spatially heterogeneous improvements in paracellular sealing that do not translate into obvious changes in global ZO-1 patterning or bulk F-actin distribution. The lack of effect on any marker in the IL-1β model further supports the idea that CBN's junctional actions are cytokine- and pathway-specific, with stronger engagement of TNF-α–related adhesion and signaling programs than of IL-1β-driven cytoskeletal responses.

Importantly, IL-1β and TNF-α are not unique to sepsis but are also rapidly induced in ischemia–reperfusion settings such as acute stroke and post-reperfusion neuroinflammation, where they contribute to BBB breakdown and cerebral edema[20–22]. Similarly, major tissue trauma and surgery trigger a systemic cytokine response characterized by early IL-1β and delayed but marked IL-6, IL-8 and TNF-α elevations, which are closely linked to postoperative complications and organ dysfunction[23]. In these settings, transient or sustained rises in circulating cytokines may similarly compromise BBB integrity and amplify neuroinflammation[24]. Consistent with brain microvascular endothelium acting as an active inflammatory effector, TNF-α and IL-1β alone produced a reproducible barrier-injury phenotype across both HBMEC donors, with sustained TEER decline and negative ΔAUC versus medium. Notably, this coupling of inflammatory activation with barrier failure is not unique to the BBB: human glomerular microvascular endothelial cells similarly increase permeability while concomitantly activating cytokine production in response to microbial and host inflammatory cues[25]. Together, these observations support the broader relevance of our findings—TNF-α/IL-1β-driven endothelial barrier dysfunction represents a shared pathway across sepsis, ischemia–reperfusion injury, and surgical/traumatic insults—suggesting that the cannabinoid responses we observed may extend beyond classical sepsis and septic encephalopathy.

The divergent responses to TNF-α and IL-1β may reflect fundamental differences in the mechanisms by which they regulate and disrupt endothelial cell homeostasis. TNF-α primarily promotes cytoskeletal contraction and junctional disassembly by activating the NF-κB and RhoA/ROCK pathways, leading to stress fiber formation and loss of VE-cadherin continuity [26, 27]. Conversely, IL-1β activates a distinct signaling profile dominated by MyD88–IRAK–p38/JNK cascades, which amplify inflammatory gene expression and cytokine release rather than directly inducing cytoskeletal stress[28, 29]. The strong

barrier-protective effect of CBN under TNF-α stimulation, but not IL-1β, suggests that CBN may preferentially interfere with the NF-κB– or RhoA/ROCK-mediated cytoskeletal contractility pathway. Differential receptor expression and signal coupling may also underlie cytokine specificity. Cannabinoid receptors, including CB1, CB2, PPARγ, and TRPV channels, are variably expressed among endothelial populations and can differentially regulate anti-inflammatory and cytoprotective signaling[30, 31]. The selective stabilizing of barrier function of CBN against TNF-α-mediated barrier injury may involve CB2 activation. Previous studies have demonstrated that stimulation of CB2 with the synthetic agonist PM289 improves brain endothelial barrier integrity, reduces inflammatory cytokine release, and accelerates endothelial repair following cytokine challenge, supporting a CB2-dependent mechanism underlying cannabinoid-mediated barrier protection[32]. PPARγ activation exerts anti-inflammatory and junction-stabilizing effects[33, 34]. Therefore, the selective efficacy of CBN under TNF-α conditions may be attributed to its partial agonism at CB2 and PPARγ, both of which negatively regulate NF-κB–driven inflammation.

Collectively, these findings position CBN as a promising modulator of TNF-α–driven endothelial injury, capable of preserving BBB integrity and suppressing cytokine amplification. However, its limited effect under IL-1β stimulation highlights the importance of the cytokine context and receptor heterogeneity. Further studies integrating VE-cadherin imaging and multi-omics profiling are warranted to elucidate the molecular targets and signaling networks through which CBN exerts selective barrier-protective actions. Ultimately, these insights could help to inform the rational design of cannabinoid-based interventions to mitigate BBB dysfunction during systemic inflammation and sepsis-associated encephalopathy.

Although lipopolysaccharide (LPS) is commonly used to induce endothelial inflammation, we intentionally employed TNF-α and IL-1β to model the cytokine-driven barrier dysfunction. LPS acts through Toll-like receptor 4 (TLR4) on immune cells, leading to the secondary release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[35, 36]. LPS can also activate endothelial cells via TLR4. Therefore, LPS directly activates endothelial cells via interaction with endothelial TLR4, and indirectly by inducing the release of cytokines, such as TNF-α and IL-1β. In contrast, direct stimulation with TNF-α and IL-1β provides a more defined model of cytokine-mediated endothelial injury, allowing us to specifically evaluate how cannabinoids modulate canonical inflammatory pathways, such as NF-κB, RhoA/ROCK, and MAPK (p38/JNK) signaling in human brain microvascular endothelial cells (HBMEC). These cytokines are widely recognized as key mediators of BBB disruption in systemic inflammation and sepsis-associated encephalopathy[15, 37]. Moreover, HBMEC express relatively low levels of TLR4 compared to immune cells, resulting in a weaker direct response to LPS[38]. Thus, the use of TNF-α and IL-1β enables a clearer mechanistic interpretation of how cannabinoids, particularly CBN, influence endothelial barrier integrity through cytokine-specific signaling mechanisms.

The endothelial barrier stabilizing effects of CBN under TNF-α activation suggests that it interferes with contractile signaling and/or stabilizes endothelial junctional architecture. In contrast, the donor-dependent response under IL-1β implies that baseline differences between HBMEC donors - such as CB1/CB2 expression, engagement of non-canonical targets (TRP channels, PPARs, GPR55), redox status,

or junctional protein abundance - may shift CBN's net effect from protective to neutral or even detrimental. In other words, CBN appears to be a context-sensitive modulator whose efficacy is shaped by both the upstream cytokine and the intrinsic endothelial phenotype.

Methodologically, the multimodal design of this study is a major strength. Continuous TEER monitoring captured dynamic barrier trajectories, and normalization at 10 h—aligned with peak cytokine effects - allowed derivation of an integrated functional readout (ΔAUC). VE-cadherin (and ZO-1) immunofluorescence microscopy anatomically anchor these functional changes: conditions that improve TEER (e.g., CBN under TNF-α) are expected to maintain continuous, linear junctional staining, whereas permeability-enhancing conditions (e.g., high-dose CBG or CBD under IL-1β) should exhibit junctional gaps, discontinuities, or internalization. Parallel IL-6/IL-8 measurements help distinguish barrier changes driven primarily by cytoskeletal/junctional mechanisms from those driven by inflammatory amplification: protective CBN conditions coincide with lower cytokine release, whereas disruptive CBD/CBG conditions are associated with higher IL-6/IL-8. Correlating ΔAUC with quantitative junction metrics (e.g., continuity index, line-scan intensity) and cytokine outputs will sharpen causal inference.

Although previous studies have described the vascular actions of cannabinoids, including CBD-associated improvements in endothelial function[18], the comparative effects of minor cannabinoids under defined inflammatory stimuli remain unclear. Reviews of cannabinoid biosynthesis further emphasize that many minor phytocannabinoids remain insufficiently characterized in terms of bioactivity[17]. Our study fills this gap by demonstrating that CBN uniquely and reproducibly protects the BBB from TNF-α–induced dysfunction while concurrently reducing inflammatory cytokine release. These findings align with emerging evidence that cannabinoids influence endothelial responses in context-dependent ways. Thus, our results provide novel mechanistic insight into the endothelial actions of minor cannabinoids and identify CBN as a credible candidate for mitigating BBB injury during systemic inflammation.

Several limitations guide interpretation and future directions. First, a monoculture HBMEC system cannot recapitulate the full neurovascular unit (astrocytes, pericytes, microglia) or hemodynamic shear. Therefore, follow-up work in co-culture, BBB-on-chip, or flow-based models is needed. Second, donor heterogeneity was evident, particularly for CBN under IL-1β. Increasing donor numbers, stratifying by sex and age, and profiling baseline receptor and junctional protein expression should help identify predictors of response. Third, mechanistic specificity remains to be defined. Pharmacological dissection with CB1/CB2 antagonists, TRP channel and PPAR inhibitors, together with readouts of cytoskeletal tension (p-MLC, RhoA activity), junctional turnover (p120/β-catenin dynamics), and oxidative stress/Nrf2 signaling, will clarify how each cannabinoid alters barrier integrity. Finally, the dose–response relationships, especially for CBG, were non-monotonic, highlighting the need for finer concentration mapping (pM–low μM range) and stringent vehicle controls. Analogous to the effects of the cannabinoids on endothelial activation and dysfunction, we have also found that CBN, CBG, and CBD differentially modulate the activation of sensory neurons, as assessed by Calcium imaging[39].

Clinically, circulating markers of TNF-α–driven endothelial injury such as Angiopoietin-2 and syndecan-1 are strongly associated with sepsis severity, microvascular dysfunction, and mortality, reinforcing the concept of an “endotheliopathy” endotype in which TNF-α and endothelial damage are tightly coupled[40–42]. This is consistent with extensive experimental work showing that TNF-α is a central driver of endothelial activation, tight-junction disruption, and microvascular leak in sepsis models, largely via NF-κB–dependent signaling and junctional protein loss[43, 44]. Although broad anti-TNF strategies in unselected sepsis populations have yielded mixed overall results, subgroup and meta-analytic data (for example with afelimomab) suggest potential benefit in biologically defined subsets, particularly patients with high baseline inflammatory burden (e.g., elevated IL-6)[45–47]. Together with our in-vitro data, this supports a testable hypothesis that CBN—or CBN-like compounds—might be most effective in patients with a “TNF-high, endotheliopathy-high” inflammatory endotype characterized by elevated TNF-α, soluble TNF receptors, and endothelial injury markers such as Ang-2 and syndecan-1[48, 49]. Future studies integrating plasma cytokine profiling, endothelial biomarkers, and in-vivo or advanced in-vitro BBB readouts could evaluate whether CBN-based adjunctive therapy selectively stabilizes the BBB and mitigates cerebrovascular injury in these TNF-α–driven clinical phenotypes.

Conclusions

In our HBMEC model, CBN reproducibly attenuated TNF-α–induced barrier dysfunction and suppressed downstream IL-6 and IL-8 release, whereas its effects under IL-1β were weak and heterogeneous. This suggests that CBN preferentially engages TNF-α–linked endothelial pathways. From a translational standpoint, our findings position CBN as a promising candidate for TNF-α–dominant inflammatory states, such as systemic inflammation and sepsis-associated encephalopathy.

Abbreviations

ANOVA
Analysis of variance
ΔAUC
Reduction in area under the curve
BBB
blood-brain barrier
CBN
Cannabinol
CBG
Cannabigerol
CBD
Cannabidiol
ECIS
Electric cell-substrate impedance sensing
HBMEC

Human brain microvascular endothelial cells
IL
1β-Interleukin-1beta
IL
6-Interleukin 6
IL
8-Interleukin 8
MeOH
Methanol
TEER
Transendothelial electrical resistance
THC
Δ-9-tetrahydrocannabinol
TNF
α-Tumor necrosis factor alpha
VE
cadherin-Vascular endothelial-cadherin
ZO
1-Zonula Occludens-1

Declarations

- **Ethics approval and consent to participate:** Not applicable
- **Consent for publication:** Not Applicable
- **Availability of data and materials:** The datasets used during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests
- **Funding:** This work was funded by NIH/NCCIH R01AT010757 (Hellman PI/Schumacher MPI) and the UCSF Department of Anesthesia and Perioperative Care
- **Authors' contributions:** SQ performed experiments, analyzed and interpreted the data, wrote the initial draft of and edited the paper. FH performed cytokine analyses, analyzed and interpreted the data, and contributed to the writing and editing of the paper. NN optimized assays to measure endothelial permeability, performed permeability experiments, and analyzed data. KT optimized and performed endothelial permeability assays and analyzed data. MS participated in the conception and planning of the study, and in the critical appraisal of results and editing of the paper. IMB participated in experiments testing effects of cannabinoids on endothelial cell inflammation. EL contributed to the conception and implementation of the experiments on cannabinol and to the analysis of endothelial inflammation. FX contributed to the conception of the project, optimized endothelial permeability and inflammation assays and immunofluorescence microscopy to visualize

endothelial cells. JH conceived of the study, provided oversight for all of the experiments, analyzed and interpreted data, and edited the paper. All authors read and approved the final manuscript.

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Figures

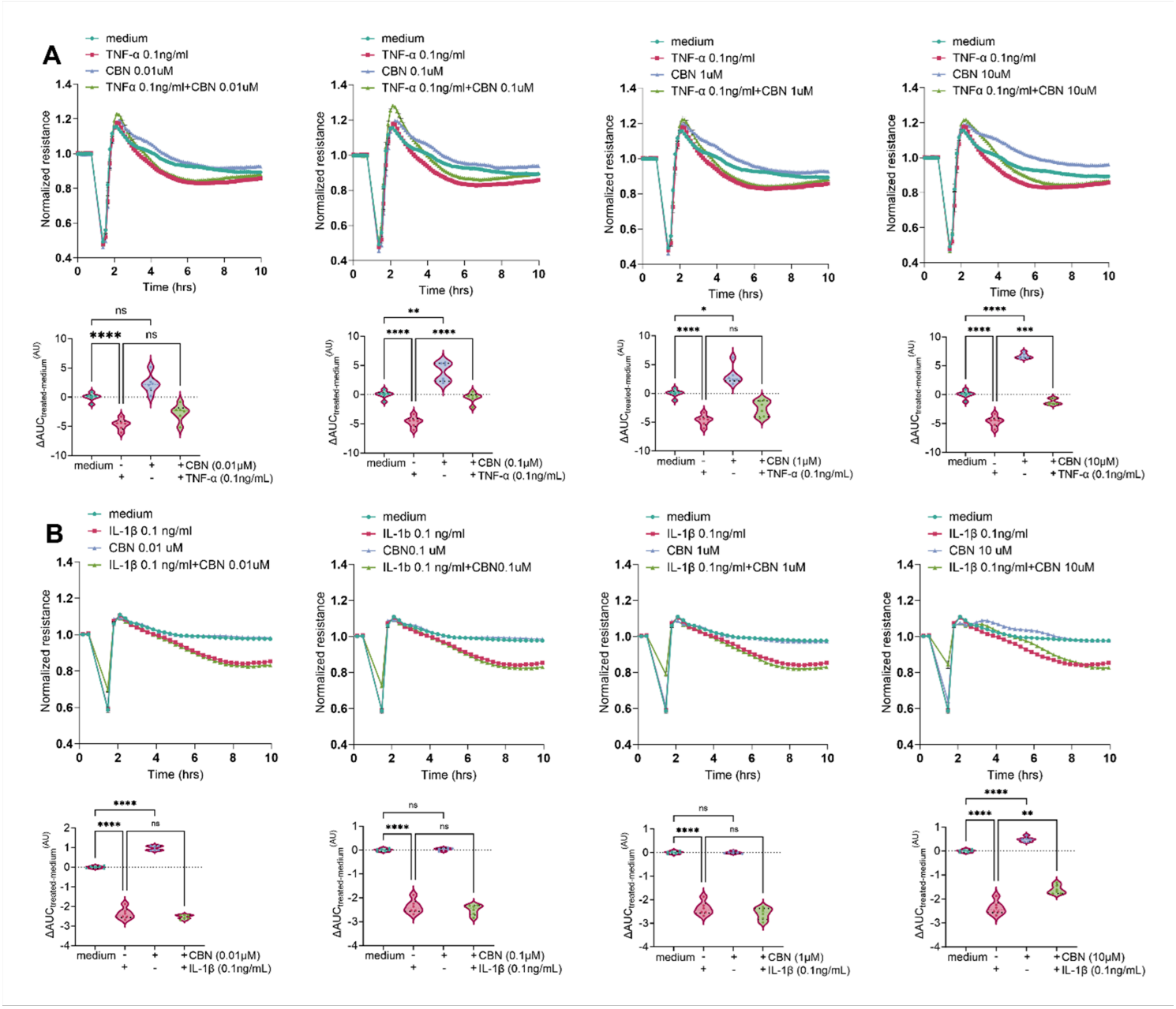

Figure 1

CBN reduces TNF-α and IL-1β–induced permeability of Donor A HBMEC.

HBMEC (Donor A) were activated with TNF-α or IL-1β (0.1 ng/mL) for 10 h in the presence or absence of CBN (0.01–10 μM). Treatment with TNF-α substantially reduced normalized resistance (TEER) and ΔAUC vs. medium alone, indicating that TNF-ahas strong barrier-disrupting effects. CBN significantly improved ΔAUC at 0.1 and 10 μM, but not at 0.01 or 1 μM. Under IL-1β, CBN at 10 μM produced a modest attenuation, while 0.01–1 μM had no effect. Donor B showed similar directional trends with a higher effective concentration under TNF-α and a shifted active dose under IL-1β (see Supplementary Figure S1). Data are mean ± SEM; one-way ANOVA with multiple comparisons. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant.

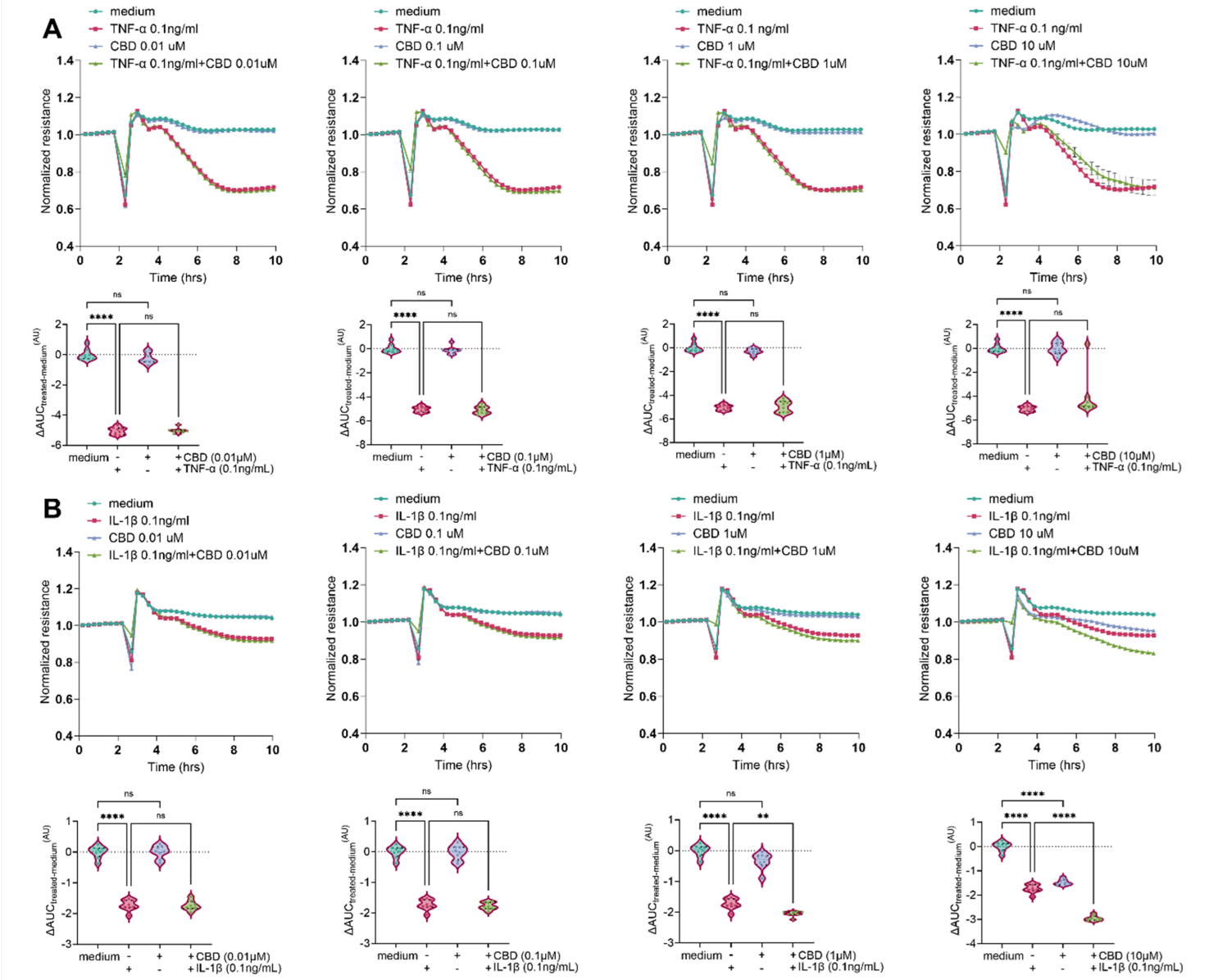

Figure 2

CBD does not abrogate TNF-α- or IL-1β-induced barrier dysfunction of Donor A HBMEC. HBMEC from Donor A were activated with TNF-α or IL-1β (0.1 ng/mL) in the presence or absence of CBD (0.01–10 μM) for 10 h. CBD did not modify TNF-α–induced reductions in resistance at any concentration. Under IL-1β, CBD concentrations of 1 and 10 μM CBD augmented barrier dysfunction. HBMEC from Donor B displayed a similar pattern, except that CBD at a concentration of 10 μM reduced TNF-α-induced barrier dysfunction, shown in Supplementary Figure 2. Data = mean ± SEM; one-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant.

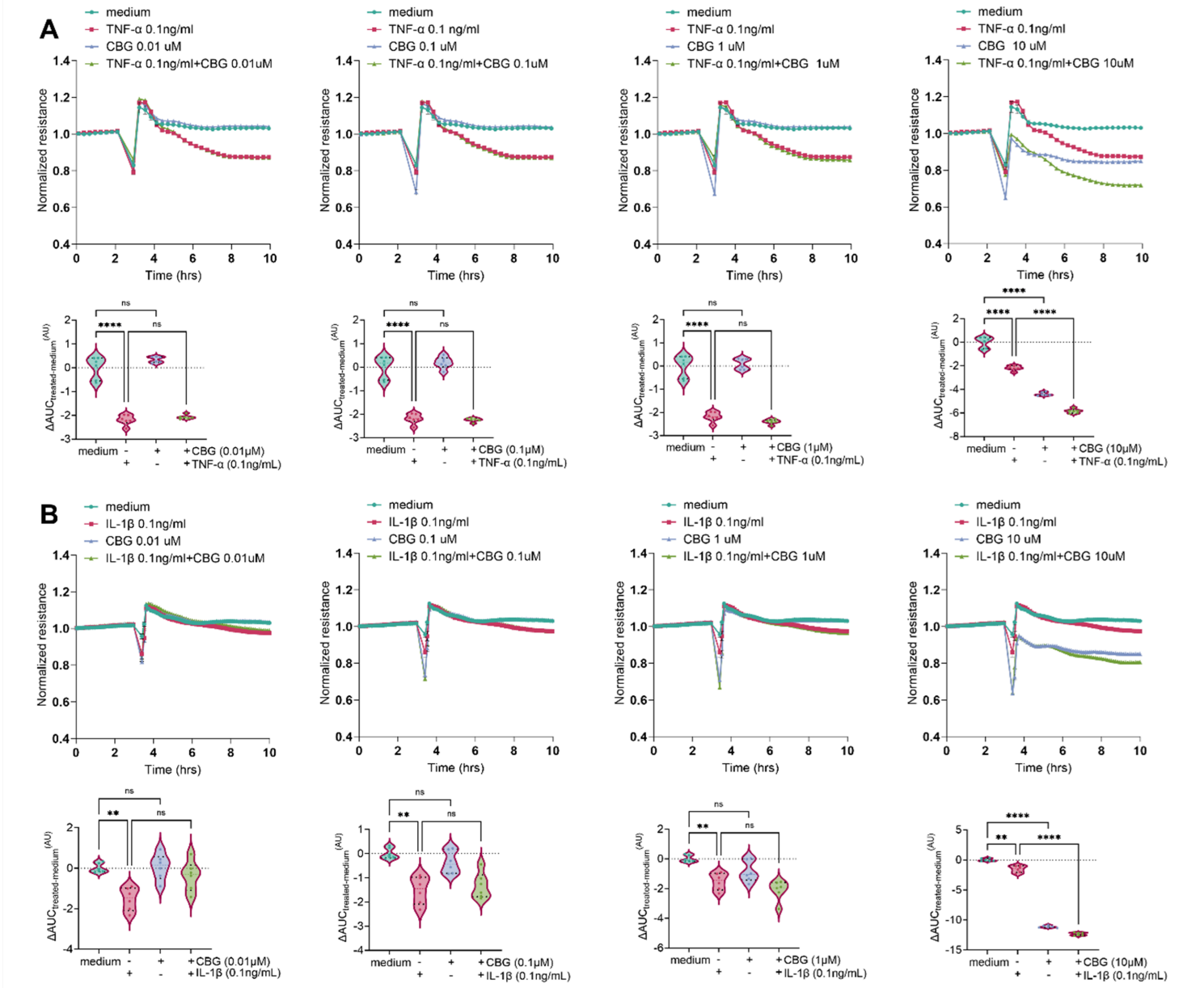

Figure 3

CBG augments TNF-α- or IL-1β-induced dysfunction of Donor A HBMEC.

HBMEC from Donor A were treated with TNF-α or IL-1β (0.1 ng/mL) for 10 h in the presence of CBG (0.01–10 μM). Under TNF-α, CBG at 0.01–1 μM did not alter the decline in normalized resistance or ΔAUC relative to TNF-α alone, whereas 10 μM CBG further reduced ΔAUC, indicating enhanced barrier dysfunction. Under IL-1β, CBG again showed no protective effect at 0.01–1 μM and 10 μM CBG produced the most pronounced worsening of barrier integrity. Donor B displayed a similar dose-dependent detrimental pattern, with 10 μM CBG consistently aggravating TNF-α– and IL-1β–induced permeability (see Supplementary Figure 3). Data represent mean ± SEM; one-way ANOVA with multiple comparisons. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant.

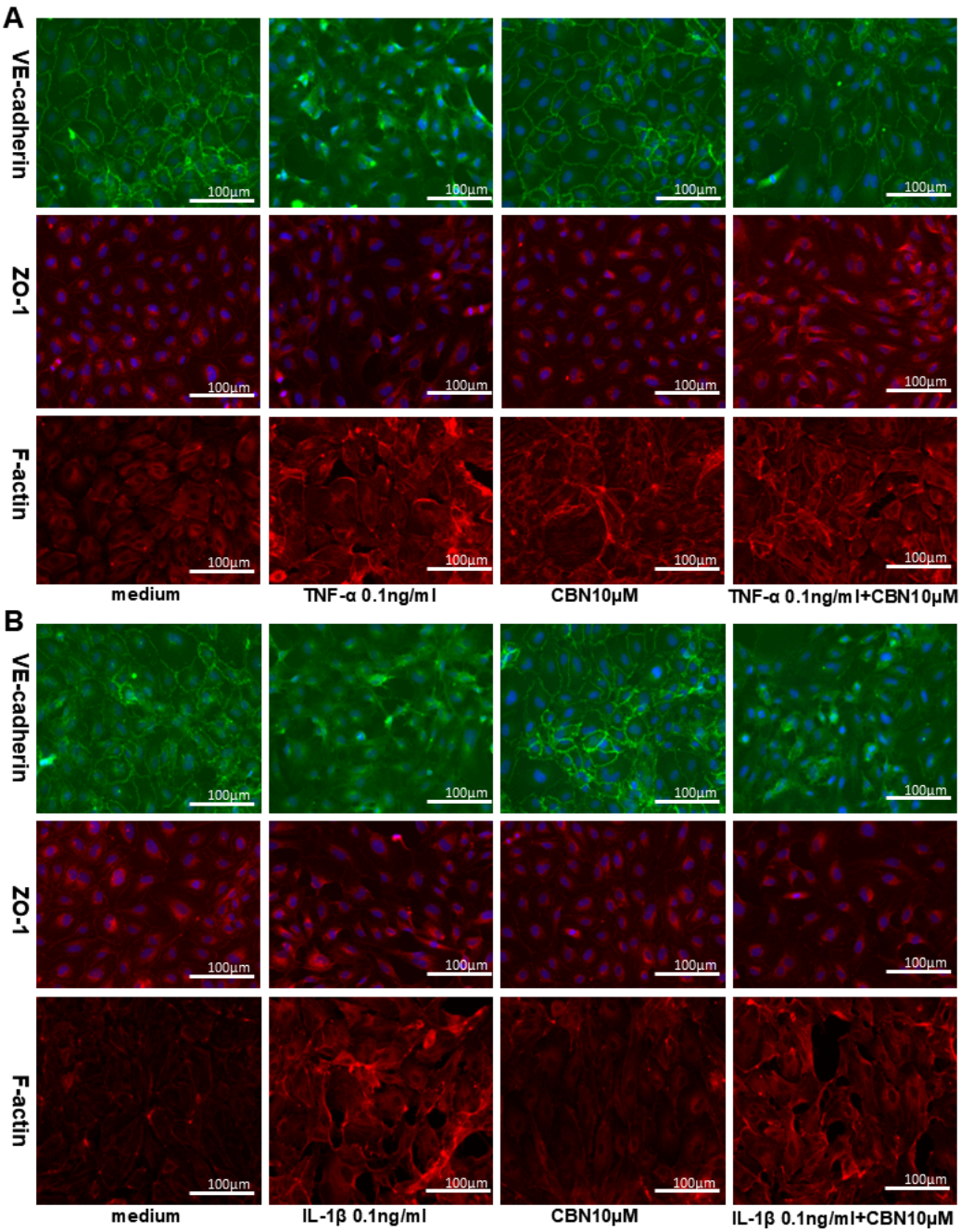

Figure 4

CBN attenuates TNF-α but not IL-1β-induced dysregulation of cell-cell junctions of Donor A HBMEC.

(A) TNF-α-Induced Permeability. Immunofluorescence staining of VE-cadherin, ZO-1, and F-actin in HBMEC treated with TNF-α (0.1 ng/mL) and/or CBN (10 μM). (B) IL-1β-Induced Permeability.

Immunofluorescence staining of VE-cadherin, ZO-1, and F-actin in HBMEC treated with TNF-α (0.1 ng/mL) and/or CBN (10 μM). Scale bars = 100 μm.

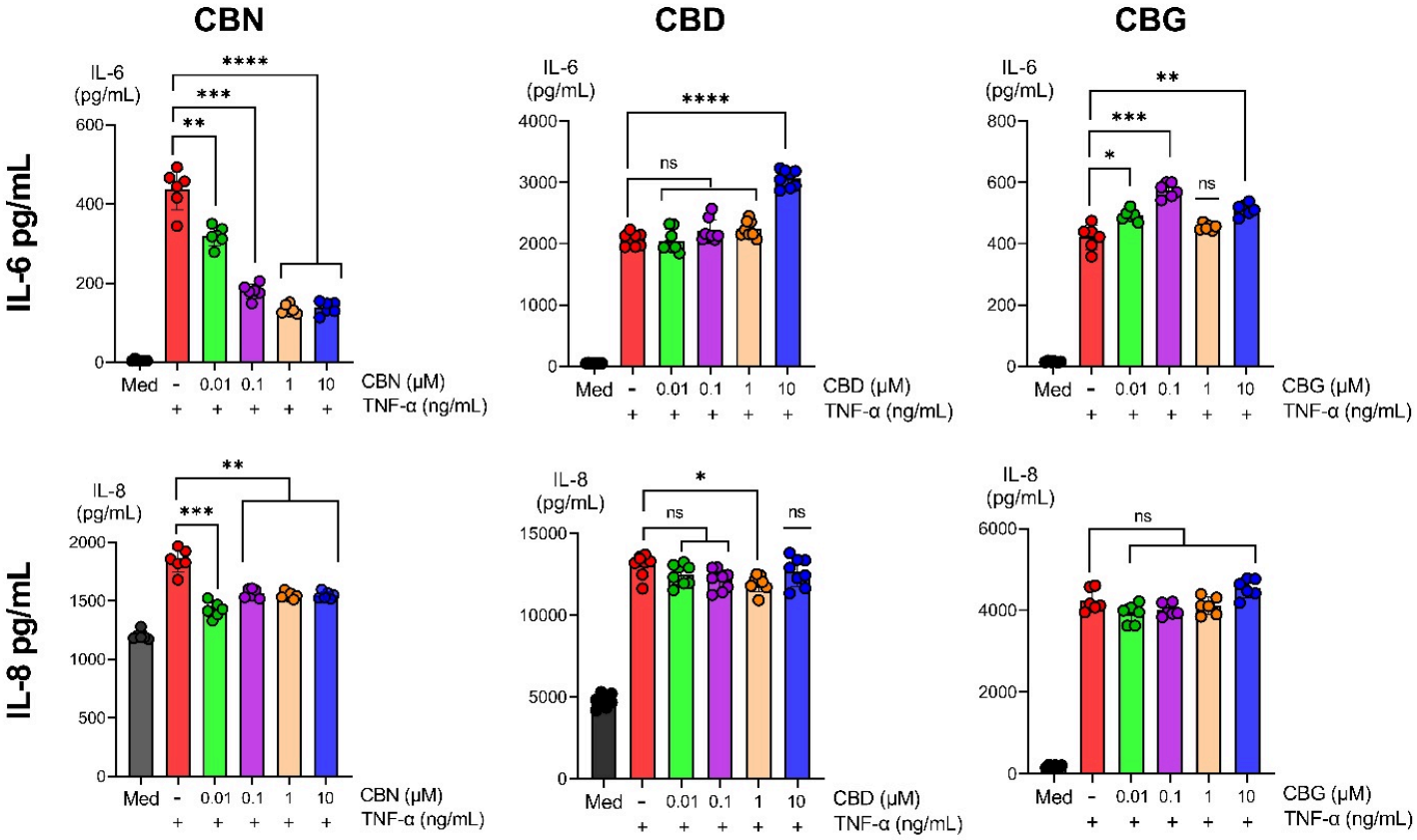

Figure 5

Cannabinoids modulate TNF-α–induced IL-6 and IL-8 secretion by Donor A HBMEC.

HBMEC were treated with TNF-α (0.1 ng/mL) together with CBN, CBG, or CBD (0.01–10 μM) for 10 h. CBN significantly reduced both IL-6 and IL-8 at all concentrations tested. CBG augmented IL-6 at several concentrations, but did not affect IL-8. CBD strongly induced IL-6 at 10 μM, and slightly reduced IL-8 at 0.1 μM. Data are mean ± SEM. One-way ANOVA with multiple comparisons. **p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*; ns = not significant.

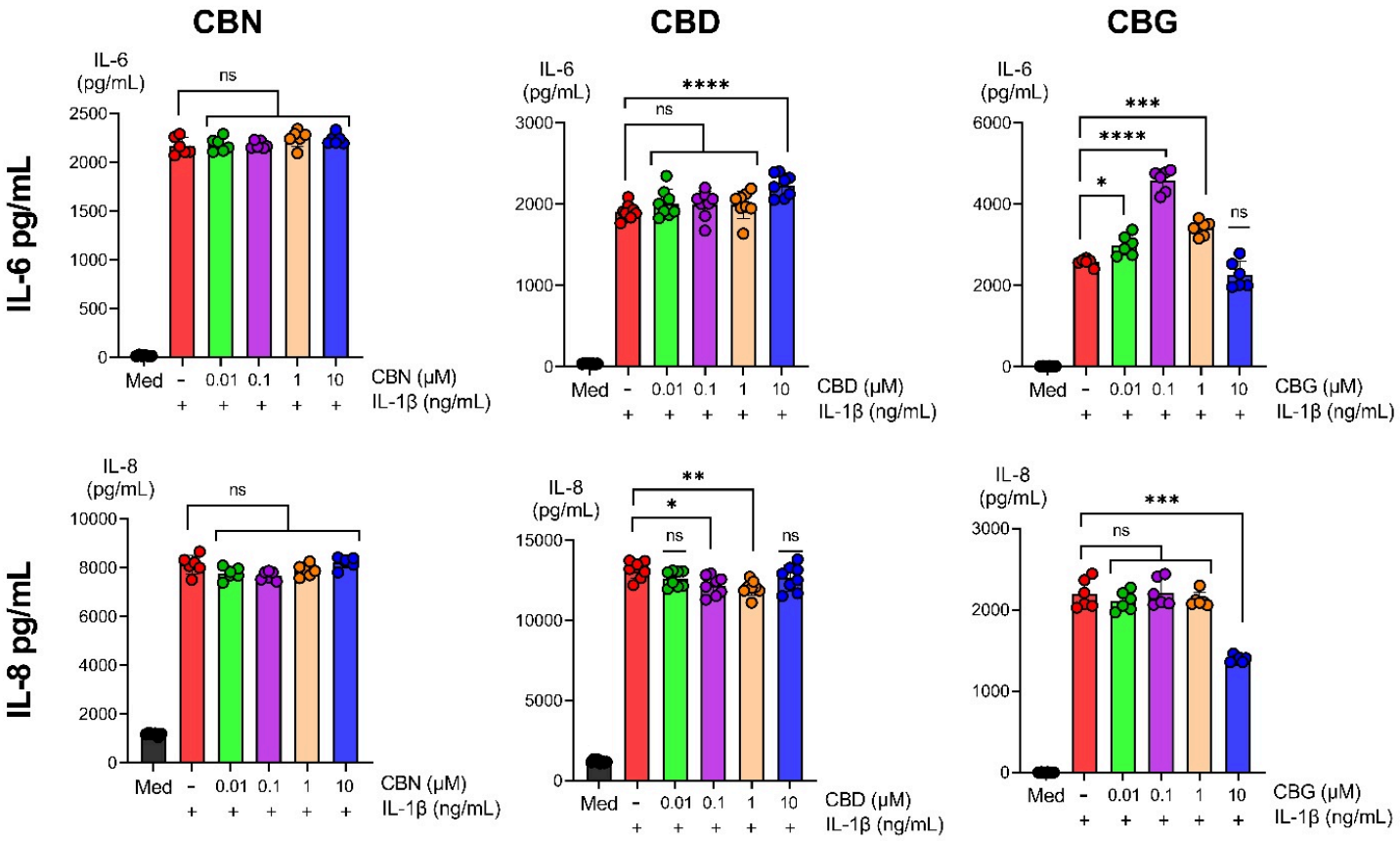

Figure 6

Cannabinoids modulate IL-1β–induced IL-6 and IL-8 secretion by Donor A HBMEC.

HBMEC were stimulated with IL-1β (0.1 ng/mL) for 10 h in the presence of CBN, CBG, or CBD (0.01–10 μM). CBN did not alter IL-6 or IL-8 at any dose. CBG increased IL-6 at 0.01–1 μM and reduced IL-8 at 10 μM. CBD increased IL-6 at 10 μM and modestly reduced IL-8 at 0.01–0.1 μM. Data are mean ± SEM; one-way ANOVA. **p < 0.05, **p < 0.01, **p < 0.001*, ns = not significant.

Supplementary Files

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- SupplementaryFigures15withLegendsFeb26.docx