frontiers in Pharmacology

TYPE Original Research
PUBLISHED 03 November 2026
DOI 10.3389/fphar.2026.1900376

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RECEIVED 04 June 2026
REVISED 06 October 2026
ACCEPTED 13 October 2026
PUBLISHED 03 November 2026

CITATION
Oostwouder C-J, Vos K, Merkus MR, Tolsma CS, de Smit MB, Telders T, van Deursen DFA, Bouwman FH, Twisk JWR, Hollmann MW and van Kalken CK (2026) Lidocaine modulates interleukin-1β and interferon-α2 secretion in LPS with ATP stimulated human peripheral blood mononuclear cells.
Front. Pharmacol. 17:1900376.
doi: 10.3389/fphar.2026.1900376

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Lidocaine modulates interleukin-1β and interferon-α2 secretion in LPS with ATP stimulated human peripheral blood mononuclear cells

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Introduction: Local anesthetics are canonically used for analgesia and anti-arrhythmic therapy, but increasing evidence indicates additional anti-inflammatory properties, including modulation of purinergic signalling. A recent retrospective observational cohort study indicated that subcutaneous lidocaine–Hydroxypropyl-β-cyclodextrin (HP-β-CD) was associated with sustained improvements in quality of life in patients with neuropathic pain in post-COVID, but the underlying mechanism remains to be established. The aim of this study was to determine whether lidocaine and articaine, alone or in combination with HP-β-CD, modulate in vitro immune cell activation and cytokine secretion in human peripheral blood mononuclear cells (PBMCs).

Methods: PBMCs were stimulated using phytohemagglutinin (PHA), lipopolysaccharide (LPS), or LPS combined with adenosine triphosphate (ATP) to engage P2X-associated inflammatory pathways, and compounds were applied both prior to and following stimulation. Cytokine secretion was quantified using a 13-plex immunoassay and cell activation was assessed by flow cytometry.

Results: Lidocaine-containing formulations attenuated cytokine secretion most prominently under conditions engaging ATP-dependent signaling, reducing interleukin-1β and interferon-α2, with interleukin-1β suppression being the most consistent effect across stimulation conditions. Articaine showed less consistent immunomodulatory effects than lidocaine, which may reflect its previously reported lack of purinergic receptor inhibition, while HP-β-CD alone did not influence immune activation or cytokine release under any condition.

Discussion: Together, these findings show that lidocaine-containing treatments can modulate cytokine responses in human PBMCs, particularly under LPS with ATP stimulation. These findings identify lidocaine as a modulator of ATP-dependent inflammatory signaling.

KEYWORDS

interferon-α2, interleukin-1β, lidocaine, local anesthetics, peripheral blood mononuclear cells, post-covid condition, purinergic signaling

Introduction

Local anesthetics are canonically used for analgesia and anti-arrhythmic therapy, but growing evidence indicates that they also possess anti-inflammatory properties (Hollmann et al., 2001; Wright et al., 2008; Hermanns et al., 2019). Among these agents, lidocaine is the most extensively studied and has demonstrated immunomodulatory effects, including inhibition of the purinergic receptor function and suppression of pro-inflammatory cytokine release (Okura et al., 2015; Hermanns et al., 2019; Oostwouder et al., 2026). An electrophysiological study further demonstrated that lidocaine also inhibits the potassium channels Kv1.3 and KCa3.1 (Oostwouder et al., 2026).

Clinical experience indicates that lidocaine can shorten hospital stays and is a promising anti-inflammatory compound (Herroeder et al., 2007; Hollmann et al., 2021). The anti-inflammatory properties of lidocaine were further demonstrated in a recent retrospective observational cohort study, which indicated that subcutaneous lidocaine–Hydroxypropyl-β-cyclodextrin (HP-β-CD) was associated with sustained improvements in symptoms and health-related quality of life in patients with neuropathic pain in post-COVID (Oostwouder et al., 2025). The effects were hypothesized to result from lidocaine's anti-inflammatory properties. Persistent inflammatory signaling, including elevated IL-1β and inflammasome activation, has been reported in post-COVID syndrome and may contribute to its sustained symptomatology, providing a pathophysiological rationale for targeting ATP/P2X-dependent immune pathways in this population (Takamatsu et al., 2026).

HP-β-CD is a widely used excipient and can indirectly interfere with receptor signaling (Gould and Scott, 2005; Loftsson and Brewster, 2010; Matassoli et al., 2018; Pirvu et al., 2025; Oostwouder et al., 2026). However, unlike lidocaine, it does not modulate purinergic receptors or Kv1.3- and KCa3.1 channel signaling, nor does it alter the inhibitory potency of lidocaine at these channels (Oostwouder et al., 2026).

Articaine, which shares structural similarities with lidocaine, has also been suggested to exert anti-inflammatory effects (Rodrigues da Silva et al., 2020). In contrast to lidocaine however, articaine does not modulate purinergic receptors, solely inhibiting the potassium channels Kv1.3-, KCa3.1, and KCa1.1 (Oostwouder et al., 2026). These immunomodulatory properties of lidocaine, articaine, and HP-β-CD on the purinergic and potassium channels have so far only been investigated in a voltage clamping study (Oostwouder et al., 2026).

To investigate whether these findings translate to human immune cells, we used peripheral blood mononuclear cells (PBMCs) as a well-established *in vitro* model to evaluate drug-induced effects on cytokine secretion. PBMCs can be stimulated with phytohemagglutinin (PHA) to induce lymphocyte activation and with lipopolysaccharide (LPS) to activate innate immune signalling through Toll-like receptor 4. Furthermore, human monocytes can secrete IL-1β following LPS stimulation through an alternative NLRP3 inflammasome pathway (Gaidt et al., 2016). To activate the P2X receptors, adenosine triphosphate (ATP) can be added, inducing additional IL-1β production. Extracellular ATP additionally induces inflammasome activation in immune cells (Karmakar et al., 2016).

As lidocaine and articaine differ in their effects on purinergic receptors (Oostwouder et al., 2026), the primary aim of this study was to determine whether lidocaine and articaine, alone or in combination with HP-β-CD, modulate immune-cell activation and cytokine secretion in PBMCs following stimulation with PHA, LPS, or LPS with ATP.

Materials and methods

Donor samples, PBMC isolation, and cell culture conditions

Healthy PBMCs were isolated from five non-pooled, anonymized buffy-coat donations obtained through the standard NHS Blood and Transplant (NHSBT) London, supply service for registered clients in the United Kingdom. Because the material was supplied in anonymized form, donor-level information, including age, sex and clinical characteristics, was not available to the investigators. All buffy coats were processed on the day of receipt to ensure optimal cell viability and function. PBMCs were isolated by density-gradient centrifugation using Histopaque according to the manufacturer's protocol. Following isolation, residual erythrocytes were removed using ammonium-chloride-potassium red blood cell lysis buffer. Cell preparations were inspected by microscopy for impurities and general cell health. Isolated PBMCs were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cultures were maintained at 37 °C in a humidified incubator containing 5% CO_2. No pooling of donor samples was performed.

Stimulants and assay reagents

PHA (Sigma), LPS (Sigma), and ATP (Sigma) were used as stimulants. Propidium iodide was used for assessment of cell viability. Phosphate-buffered saline (PBS, Gibco) was used for cell washing steps.

Antibodies and detection kits

Fluorophore-conjugated anti-human antibodies against CD69, NKp46, HLA-DR, and CD80 were used according to the manufacturer's recommendations (BioLegend). CD69 was used as an early T cell activation marker, HLA-DR and CD80 as markers

associated with antigen presentation and co-stimulatory signalling, and NKp46 as an NK-cell-associated marker. Cytokine quantification was performed using the Legendplex HU Inflammation Panel 13-plex according to the manufacturer's instructions (BioLegend, Cat. 740809).

Compound preparation

The compounds used in this study were lidocaine hydrochloride monohydrate (CAS 6108-05-0, MW 288.81), articaine hydrochloride (CAS 23964-57-0, MW 284.37), and HP-β-CD (CAS 128446-35-5, MW 1541.5) all obtained from Advanced Molecular Technologies, Melbourne, Australia, who sourced these from Roquette Frères S.A. and Fagron B.V. Working solutions were prepared fresh on the day of use and diluted in plates to the indicated final concentrations.

Viability testing

PBMCs from one donor were used to determine the optimal treatment dose while preserving cell viability. Cells were subjected to varying compound concentrations. Seven treatment conditions were evaluated: (1) Lidocaine HCl 1.5 mM, (2) Lidocaine HCl 1.5 mM combined with 0.84 mM HP-β-CD, (3) Articaine 1.5 mM, (4) Articaine 1.5 mM combined with 0.84 mM HP-β-CD, (5) HP-β-CD 0.84 mM, (6) Lidocaine 0.75 mM with Articaine 0.75 mM, and (7) Lidocaine 0.75 mM with Articaine 0.75 mM and 0.84 mM HP-β-CD. The compound concentrations were selected on the basis of tolerability in PBMC viability experiments. The anaesthetic-to-HP-β-CD ratio was informed by a previously used clinical formulation for post-COVID neuropathic pain (Oostwouder et al., 2025), but the absolute experimental concentrations were not selected to reproduce measured local tissue or systemic exposure.

In vitro cell stimulation and treatment protocols

PBMCs of four donors were seeded in a 96-well plate at 2×10^5 cells per well in 200 µL RPMI1640 media containing 10% FBS and 1% PS.

For pre-treatment experiments, PBMCs were exposed to the indicated compound formulations for one hour before stimulation. Cells were then stimulated with PHA (10 µg/mL) or LPS (100 ng/mL) in the continued presence of the compounds. For the LPS with ATP condition, PBMCs were exposed to ATP (2 mM) for the final 30 min.

For post-stimulation experiments, the PBMCs were exposed to the indicated compound formulations one hour after stimulation. Post-stimulation treatment was included as an exploratory model of intervention after inflammatory activation had been initiated.

Cell culture supernatants were collected 18–20 h after the initial stimulation and stored at −80 °C until cytokine analysis. Following supernatant collection, PBMCs were washed with PBS and processed immediately for flow cytometric analysis. Control conditions included unstimulated and untreated cells, stimulated but untreated cells, and unstimulated cells treated with the compound formulations.

Flow cytometry

The PBMCs were prepared for flow cytometry to assess the expression of cell surface markers on lymphoid and myeloid cells. PBMCs were stained with fluorophore-conjugated antibodies for human CD69, NKp46, HLA-DR, and CD80. Propidium iodide was included to determine cell viability. Up to 5,000 events were acquired within the PI-negative gated population. For gating control and fluorescence compensation, unstained cells and single color controls for each fluorescence channel were acquired separately using identical instrument settings. During analysis, acquired cells were first gated in the side scatter versus forward scatter plot to identify the target cell population followed by gating on PI-negative populations to exclude non-viable cells. The percentage of cells positive for each cell surface receptor and mean fluorescence intensity for each marker were measured. All acquired data were analyzed using GuavaSoft software (4.5).

Cytokine multiplex measurement

Cytokine quantification was performed using the Legendplex HU Inflammation Panel 13-plex according to the manufacturer's instructions (BioLegend, Cat. 740809). The panel included interleukin (IL)-1β, interferon (IFN)-α2, IFN-γ, tumor necrosis factor (TNF-α), C-C motif chemokine ligand (CCL2/MCP-1), IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33. Briefly, collected supernatants were incubated with capture bead mixtures, followed by detection antibodies and streptavidin-phycoerythrin. Mean fluorescence intensity values were converted to cytokine concentrations (pg/mL) using kit-specific standard curves and the software provided by the manufacturer (BioLegend). All measurements were performed on supernatants collected 18–20 h after stimulation.

Data analysis and statistical method

All statistical analyses were performed using Python (version 3.13) with statsmodels (version 0.14.5), numpy (version 2.3.4), and pandas (version 2.3.3). Data visualization was performed in RStudio (version 4.5.2) using the ComplexHeatmap package (version 2.27.0).

Stimulation assays were analyzed using linear mixed-effects models applied to log-transformed data, with stimulant included as a fixed effect and donor as a random intercept. For activation experiments, treatment and its interaction with stimulant were added as additional fixed effects. Statistical significance was assessed using *post hoc* Wald t-tests on fixed effects, with significance set at $p < 0.05$. p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate correction. False discovery rate correction was applied separately for each experiment type: stimulation-only, pre-stimulation treatment, and post-stimulation treatment.

Heatmaps were generated separately for each stimulation condition to visualize treatment-associated modulation patterns compared to controls. Dendrograms were included to display clustering relationships among treatment conditions and individual cytokines or markers.

Cytokines that were present at a concentration below 200 pg/mL upon stimulation were omitted from figures to avoid misinterpretation of the data.

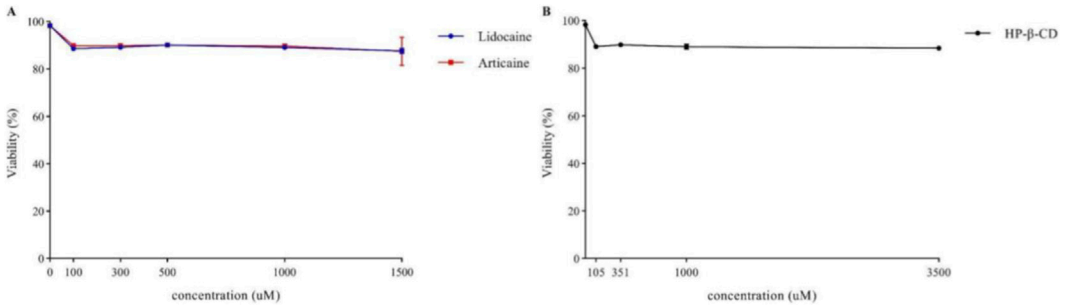


FIGURE 1
Cell viability of PBMCs following exposure to lidocaine, articaine, and HP-β-CD. All compounds used in this study were evaluated for potential cytotoxicity. PBMCs were exposed to increasing concentrations of each treatment for 24 h. The effects of lidocaine and articaine **(A)** as well as HP-β-CD **(B)** on cell viability are shown. Lidocaine and articaine did not affect viability at all concentrations tested up to 1,500 μM, and HP-β-CD showed no cytotoxic effects up to 3,500 μM. Data presented as mean with standard deviation.

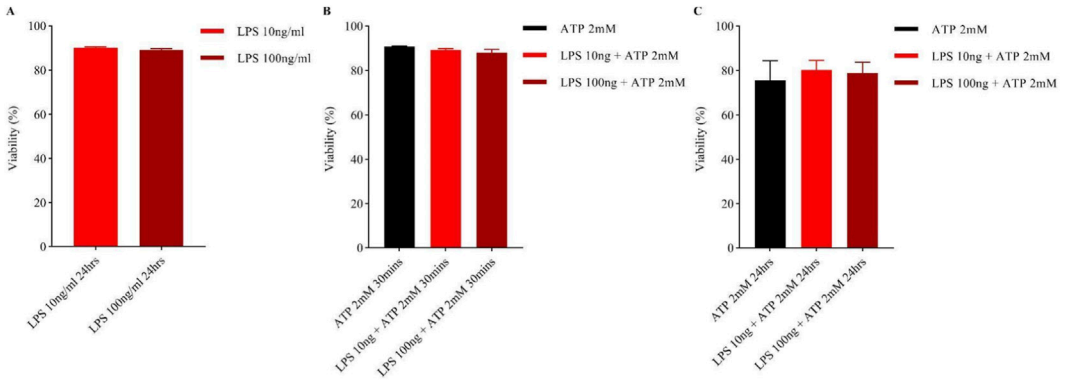


FIGURE 2
Cell viability of PBMCs following stimulation with LPS, ATP, and LPS combined with ATP. PBMCs were exposed to different stimulation conditions to assess potential cytotoxic effects. The effects of LPS alone **(A)**, LPS with the addition of ATP (2 mM) during the final 30 min **(B)**, and co-stimulation with LPS and ATP for 24 h **(C)** on cell viability are shown. LPS alone and ATP exposure during the final 30 min did not affect viability, whereas co-stimulation with LPS and ATP for 24 h resulted in reduced cell viability. Data presented as mean with standard deviation.

Results

PBMC viability

To establish appropriate treatment concentrations, PBMC viability was evaluated following exposure to increasing doses of lidocaine, articaine, and HP-β-CD. Both lidocaine and articaine did not affect viability up to 1.5 mM, while HP-β-CD was tolerated up to 3.5 mM (Figure 1). Based on these results, subsequent experiments were performed using 1.5 mM lidocaine or articaine. For combined treatments, lidocaine and articaine were each applied at 0.75 mM to achieve a total of 1.5 mM while avoiding potential cytotoxicity. HP-β-CD was used at a concentration of 0.84 mM, in line with the ratio of lidocaine-HP-β-CD in a formulation used clinically for neuropathic pain in post-COVID. These concentrations were used in subsequent activation-marker and cytokine experiments.

To determine stimulation conditions, PBMCs were exposed to varying concentrations of LPS or LPS combined with ATP, and viability was assessed (Figure 2). LPS alone was well tolerated at 100 ng/mL after 24 h. Addition of 2 mM ATP for 30 min did not affect viability. Co-stimulation with LPS and ATP for 30 min did not further alter viability. However, continuous exposure to ATP for 24 h reduced PBMC viability. Consequently, all subsequent experiments were conducted using 100 ng/mL LPS for 18–20 h, with 2 mM ATP added during the final 30 min for the LPS with ATP condition. The effect of PHA on PBMC viability was not assessed, as PHA at the concentrations used was not expected to exert cytotoxic effects based on in-house experimental experience.

Immune cell activation and cytokine responses following stimulation with PHA, LPS, and LPS with ATP

PBMC activation was evaluated by quantifying CD69, HLA-DR, CD80, and NKp46. PHA, LPS, and LPS in combination with ATP induced all measured cell activation markers, with representative

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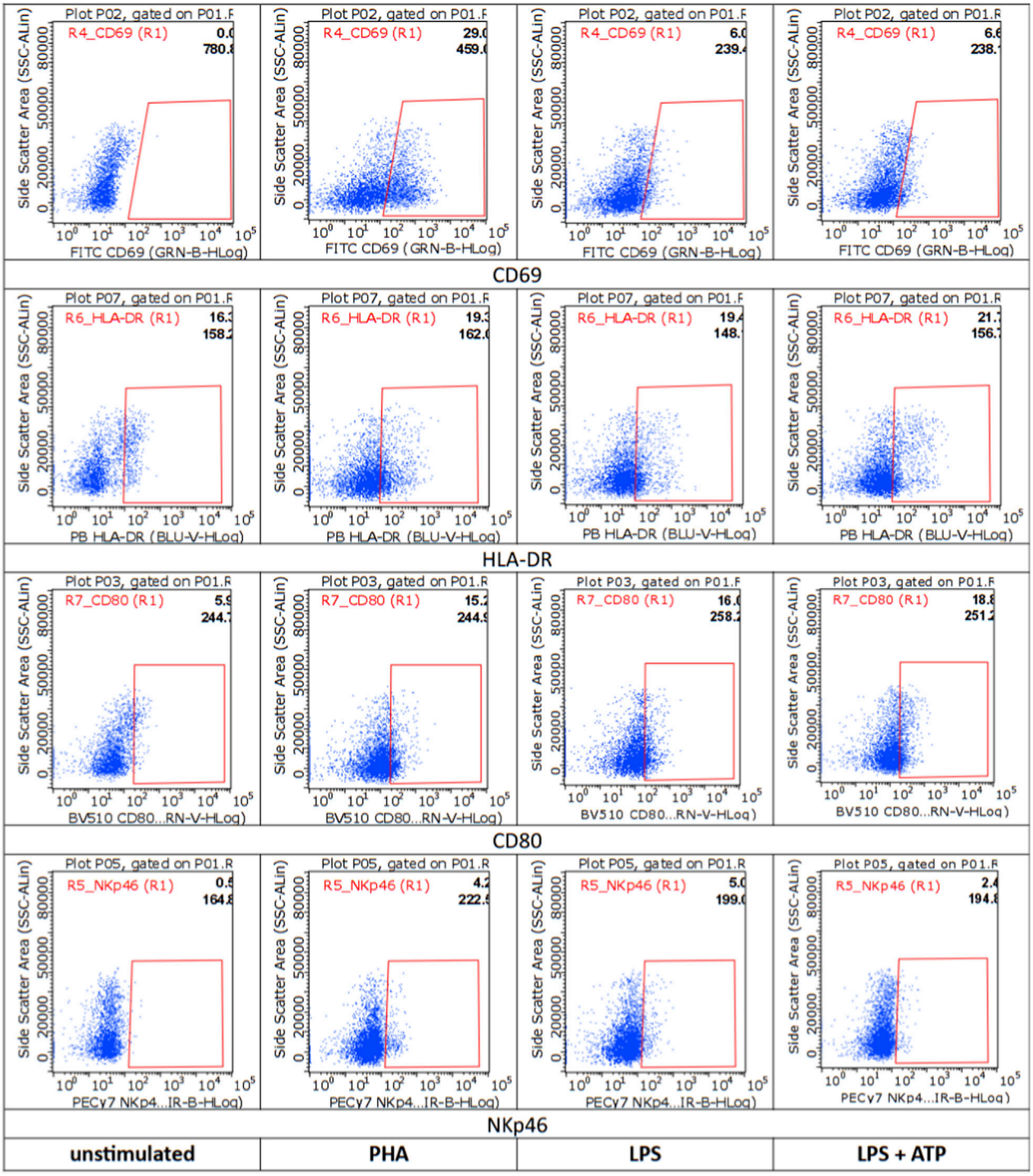


FIGURE 3
Flow cytometry of activation markers before and after stimulation PBMCs expressing the indicated activation markers (CD69, HLA-DR, CD80, and NKp46) were identified by applying predefined gates, shown in red. Representative single-donor flow cytometry plots are shown for unstimulated PBMCs and following stimulation with PHA, LPS, or LPS with ATP. Rows show the expression of CD69, HLA-DR, CD80, and NKp46, respectively.

flow cytometry plots shown in Figure 3. The most pronounced effect was the increase in CD69 expression following PHA stimulation. Fold-changes relative to unstimulated PBMCs are shown in Figure 4.

Cytokine secretion following stimulation with PHA, LPS, or LPS in combination with ATP was assessed in PBMCs from four individual donors (Figure 5). In all stimulated conditions, IL-12p70, IL-17A, IL-18, IL-23, IFN-ɣ, and IL-33 were not detected at concentrations above 200 pg/mL and were therefore excluded from further analysis. Subsequent analyses focused on the cytokines IL-1β, IL-6, IL-8, IL-10, TNF-α, MCP-1, and IFN-α2. PHA induced all measured cytokines except for IL-1β and IFN-α2. LPS and LPS in combination with ATP induced all measured cytokines.

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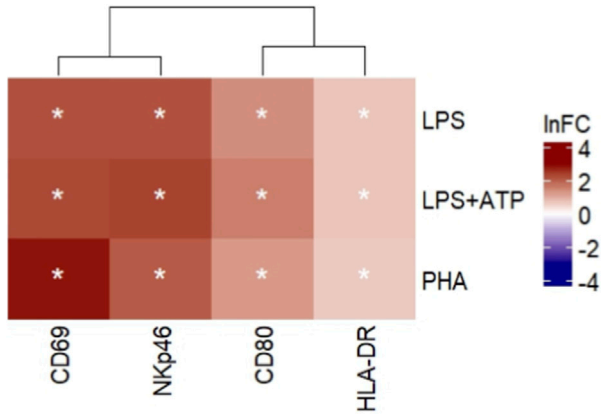


FIGURE 4
Immune cell activation response in human PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs were stimulated with PHA, LPS, or LPS with ATP, and the expression of cell activation markers was measured using flow cytometry. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate statistical significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates the similarities between marker activation.

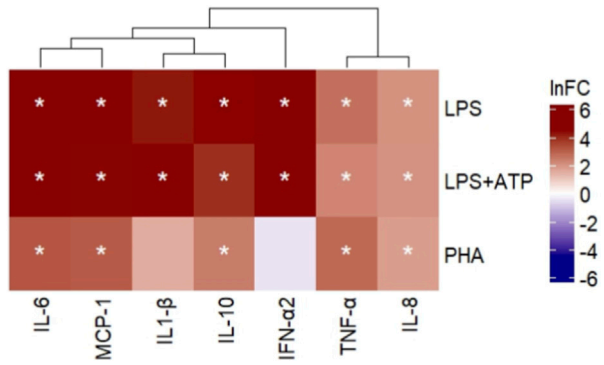


FIGURE 5
Cytokine responses in human PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs from four individual donors were stimulated with PHA, LPS, or LPS with ATP, and cytokine levels in culture supernatants 18–20 h after stimulation were quantified relative to unstimulated controls. Data were normalized and presented relative to unstimulated controls. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates similarities between the expression of the different cytokines.

Effect of lidocaine, articaine, HP-β-CD, and their combinations on cell activation

Overall, expression of CD69, HLA-DR, CD80, and NKp46 was largely unaffected by treatments across all stimuli (Figure 6). Following PHA stimulation, the treatment containing articaine and lidocaine in combination with HP-β-CD reduced NKp46 expression ($\beta = -0.45$, $p = 0.017$). Upon stimulation with LPS and LPS with ATP, articaine in combination with HP-β-CD reduced NKp46 expression ($\beta = -0.44$, $p = 0.018$; $\beta = -0.56$, $p = 0.015$).

Effect of lidocaine, articaine, HP-β-CD, and their combinations on cytokine secretion

PBMCs were treated prior to stimulation with PHA, LPS, or LPS in combination with ATP, and the effects on cytokine secretion are shown in Figure 7. Following PHA stimulation, treatment with a combination of lidocaine, articaine, and HP-β-CD reduced TNF-α ($\beta = -1.54$, $p = 0.018$) and IL-10 ($\beta = -1.91$, $p = 0.040$) concentrations. Upon LPS stimulation, all lidocaine-containing treatment groups reduced IL-1β concentrations ($\beta = -3.59$ to -2.68, $p < 0.05$). In addition, lidocaine in combination with articaine reduced IFN-α2 secretion ($\beta = -5.45$, $p = 0.010$). In contrast, formulations without lidocaine did not result in significant changes in cytokine secretion. In the LPS with ATP–stimulated condition, a broader suppression of cytokine secretion was observed following treatment, as shown in Figure 7. Most notably, lidocaine-containing formulations reduced IL-1β ($\beta = -4.64$ to -4.09, $p < 0.05$) and IFN-α2 ($\beta = -6.71$ to -5.92, $p < 0.05$) levels. Articaine reduced IL-1β secretion under these conditions ($\beta = -3.11$, $p = 0.026$). Overall, suppression of IL-1β release was the most consistent treatment-related effect observed across stimulation conditions in lidocaine-containing formulations. Across all stimulation conditions and treatments, IL-6 and IL-8 were not affected.

Effect of post-stimulation treatment on cell activation and cytokine secretion

To evaluate whether treatment effects differed if the treatment was applied after stimulation, PBMCs were treated with lidocaine, articaine, HP-β-CD, or their combinations following stimulation (Figures 8, 9). Overall, expression of CD69, HLA-DR, CD80, and NKp46 was largely unaffected by treatments across all stimuli (Figure 8). When stimulated with PHA, treatment with articaine and articaine in combination with HP-β-CD increased HLA-DR and/or CD80 expression ($\beta = 0.36$ to 0.56, $p < 0.05$). In PHA- and LPS-stimulated PBMCs, treatment with a combination of lidocaine, articaine, and HP-β-CD, reduced NKp46 expression (PHA: $\beta = -0.59$, $p = 0.032$; LPS: $\beta = -0.55$, $p = 0.043$). Stimulation with LPS in combination with ATP displayed no significant changes in cell activation.

The effects of the post-treatment application on cytokine secretion are shown in Figure 9. Overall, effects were generally less pronounced compared to the pre-treatment condition, with the most notable observation being a reduction of IL-1β and IFN-α2 in the LPS stimulated group in lidocaine-articaine-containing treatments ($\beta = -5.53$ to -2.29, $p < 0.05$). HP-β-CD alone had no effect under any post-stimulation condition.

Discussion

The primary aim of this study was to evaluate whether lidocaine and articaine, both alone and in combination with HP-β-CD, modulate human PBMC activation and cytokine secretion

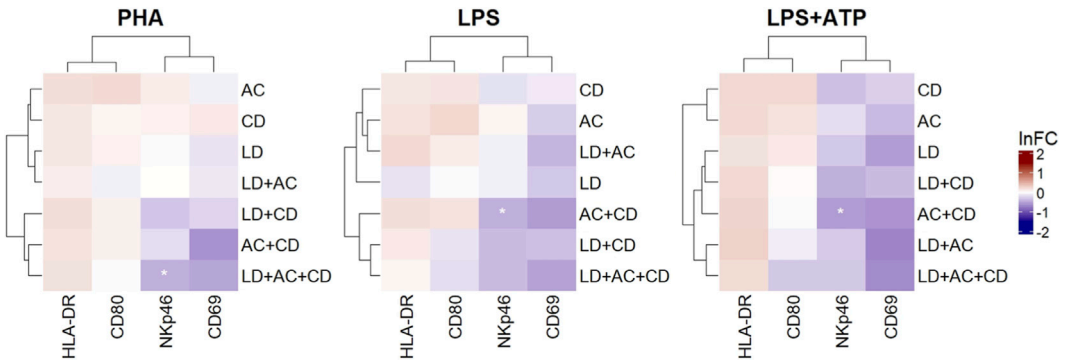


FIGURE 6
Effect of lidocaine, articaine, HP-β-CD, and their combinations on immune cell activation in human PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs from four individual donors were treated with lidocaine (LD), articaine (AC), HP-β-CD (CD), or their combinations prior to stimulation with PHA, LPS, or LPS with ATP. Activation marker expression was normalized to the respective no-treatment control for each stimulus. Data were normalized and presented relative to untreated conditions. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates similarities in marker expression.

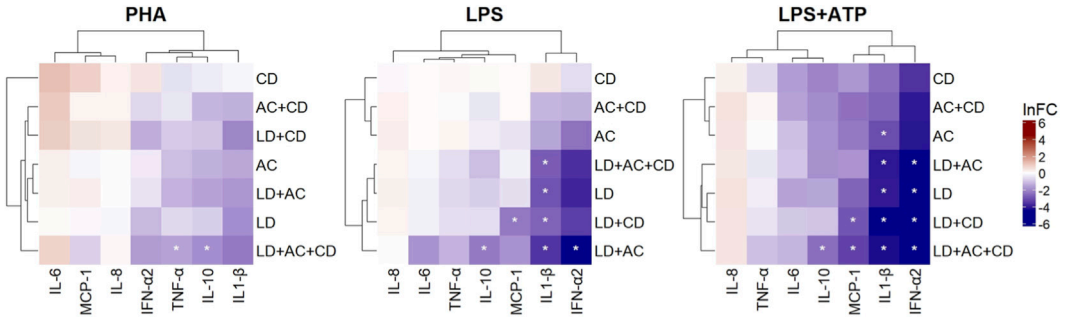


FIGURE 7
Effect of lidocaine, articaine, HP-β-CD, and their combinations on cytokine production in human PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs from four individual donors were treated with lidocaine (LD), articaine (AC), HP-β-CD (CD), or their combinations prior to stimulation with PHA, LPS, or LPS with ATP. Cytokine levels in culture supernatants 18–20 h after stimulation were normalized and presented relative to the respective no-treatment condition for each stimulus. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates similarities in cell response.

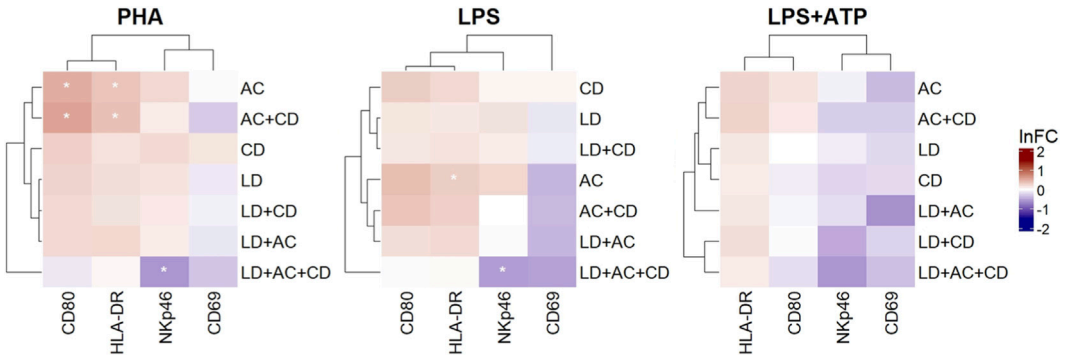


FIGURE 8
Effect of lidocaine, articaine, HP-β-CD, and their combinations on immune cell activation in PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs from four individual donors were treated with lidocaine, articaine, HP-β-CD, or their combinations after stimulation with PHA, LPS, or LPS combined with ATP. Activation marker expression was normalized and presented relative to the respective no-treatment condition for each stimulus. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates similarities in marker expression.

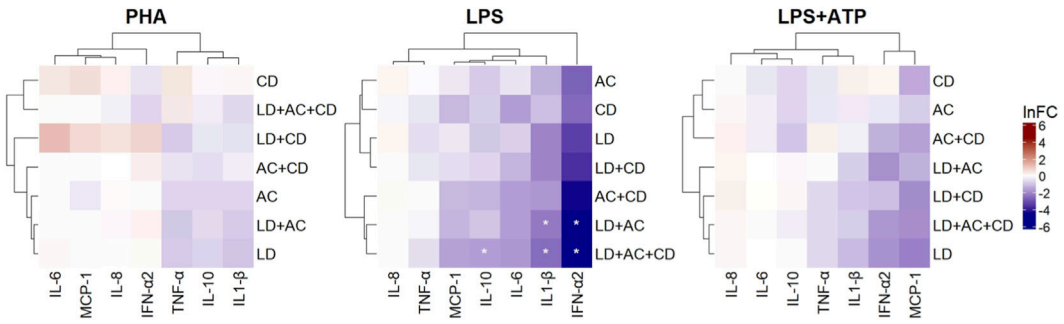


FIGURE 9
Effect of lidocaine, articaine, HP-β-CD, and their combinations on cytokine production in PBMCs stimulated with PHA, LPS, or LPS with ATP PBMCs from four individual donors were treated with lidocaine (LD), articaine (AC), HP-β-CD (CD), or their combinations after stimulation with PHA, LPS, or LPS with ATP. Cytokine levels 18–20 h after stimulation were normalized and presented to the respective no-treatment condition for each stimulus. Log-transformed data were analyzed with Linear mixed-effects models. Predicted fixed effects are shown. White asterisks indicate significance. Post hoc Wald t-tests on fixed effects, $p < 0.05$. Dendrogram indicates similarities in cell response.

following stimulation. We characterized the effects under three stimulation conditions: PHA, LPS, and LPS combined with ATP.

All stimulants increased the expression of CD69, NKp46, CD80, and HLA-DR. CD69 was used as an early T cell activation marker, NKp46 as an NK-cell-associated marker, and HLA-DR and CD80 as markers associated with antigen presentation and co-stimulatory signalling. PHA stimulation produced the most pronounced increase in CD69 expression, consistent with its expected lymphocyte-activating effect (Ceuppens et al., 1988). However, because CD3 co-staining was not performed, the observed CD69 response cannot be fully attributed to T cells.

A principal finding of this study was the reduction of IL-1β secretion by lidocaine-containing treatments, including under LPS with ATP stimulation. Previous electrophysiological studies demonstrated that lidocaine can inhibit P2X receptor function, providing a rationale for considering purinergic signalling as one possible mechanism (Okura et al., 2015; Oostwouder et al., 2026). IL-1β production under ATP stimulation is well established to depend on ATP-mediated activation of P2X receptors and subsequent inflammasome signaling (Karmakar et al., 2016) However, the present experiments did not directly assess P2X receptor expression or function. Human monocytes can also release IL-1β through LPS-driven alternative inflammasome signalling (Gaidt et al., 2016). The observed reduction in IL-1β is therefore consistent with modulation of ATP-sensitive inflammatory signalling but does not establish direct inhibition of P2X receptors in PBMCs.

The observed reduction in IL-1β may be clinically relevant, as persistent elevation of IL-1β and inflammasome-associated signaling has been reported in post-COVID syndrome (Takamatsu et al., 2026). In addition, IL-1β is increasingly implicated in neuropathic pain mechanisms and neuroimmune dysregulation (Gui et al., 2016). These observations support the potential relevance of immunomodulatory approaches targeting ATP-dependent inflammatory signaling in inflammatory and post-viral conditions.

In addition to IL-1β, lidocaine-containing formulations reduced IFN-α2 secretion under LPS and LPS with ATP stimulation. Type I interferons, including IFN-α2, play central roles in innate immune activation and antiviral responses. While the mechanistic link between lidocaine and IFN-α2 suppression is less direct than for IL-1β, these data suggest that lidocaine may exert broader regulatory effects on innate immune signaling downstream of TLR4 and purinergic receptor activation.

Articaine exhibited more limited immunomodulatory effects compared to lidocaine. The lack of consistent suppression of IL-1β by articaine under LPS with ATP stimulation is compatible with previously reported differences between articaine and lidocaine in their effects on purinergic receptor function (Oostwouder et al., 2026). Direct comparison of receptor-specific effects in immune cells will be required to determine whether this explains the observed cytokine responses.

HP-β-CD alone did not alter immune cell activation or cytokine secretion under any stimulation condition, supporting the interpretation that observed cytokine effects were driven by the local anesthetics. All treatment and stimulation conditions were well tolerated, with no significant loss of cell viability, indicating that the observed effects were not attributable to cytotoxicity.

This study was exploratory in nature, with a limited sample size and substantial inter-donor variability, which likely reduced statistical power. We prioritized comprehensive profiling across multiple conditions and stimulations. The present findings therefore provide a foundation for subsequent investigations designed with fewer experimental variables and expanded donor numbers to further validate these results.

Several methodological limitations should be noted. PBMCs are a heterogeneous cell population suited to studying general immunomodulatory effects, but future studies focusing on single immune cell populations and time-resolved sampling will be essential to delineate the cellular sources and molecular mechanisms underlying the observed effects. Cytokine measurements were obtained at a single time point, precluding assessment of temporal dynamics, and only a single compound concentration per condition was evaluated, preventing characterization of concentration-response relationships. In addition, the concentrations were selected for exploratory *in vitro* testing and should not be interpreted as representative of systemic clinical exposure. Future studies should include concentration-

response experiments and compare the experimental concentrations with clinically achievable local tissue and systemic concentrations. Furthermore, because PBMCs are heterogeneous, the cellular sources of the observed cytokine responses could not be identified in this study. In human PBMC preparations, IL-1β secretion is expected to arise predominantly from monocytes, whereas IFN-α responses are commonly associated with plasmacytoid dendritic cells (Semino et al., 2018; Schloer et al., 2025). Donor sex may also influence IFN-α responses (Schloer et al., 2025). These cellular and donor-related factors should be investigated.

In conclusion, this study extends the reported anti-inflammatory properties of lidocaine and articaine to the attenuation of cytokine production in human PBMCs. Lidocaine-containing treatments attenuated cytokine responses in human PBMCs, with the most consistent effects observed for IL-1β. The effects observed under LPS with ATP stimulation are compatible with modulation of ATP-sensitive inflammatory pathways, but receptor-specific and cell-type-resolved experiments are required to clarify the underlying mechanism.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because this study used primary human peripheral blood mononuclear cells isolated from anonymised buffy-coat donations, sourced by ImmunDNZ, and supplied by NHS Blood and Transplant, United Kingdom. No donor-identifying information was available to the investigators.

Author contributions

C-JO: Methodology, Project administration, Conceptualization, Data curation, Funding acquisition, Writing – original draft, Investigation, Writing – review and editing, Resources. KV: Methodology, Conceptualization, Investigation, Writing – review and editing, Resources, Funding acquisition. MM: Writing – review and editing, Investigation, Visualization, Validation, Data curation, Methodology. CT: Investigation, Data curation, Methodology, Software, Writing – review and editing. MdS: Writing – review and editing, Software, Data curation. TT: Writing – review and editing, Software, Data curation. DvD: Writing – review and editing, Project administration. FB: Writing – review and editing, Formal Analysis. JT: Validation, Formal Analysis, Supervision, Writing – review and editing. MH: Supervision, Writing – review and editing. CvK: Funding acquisition, Investigation, Writing – review and editing, Resources, Methodology, Conceptualization.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was funded by Remicine Biosciences B.V., the Netherlands. The funder had no role in data collection, analysis, or interpretation.

Acknowledgments

We thank Masih-ul Alam, Rawshan Choudhury, and Robert-Jan Lamers (Immundnz, United Kingdom) for performing the assays and providing valuable guidance throughout the project.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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