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Dynamical changes in the expression of GABAergic and purinergic components occur during the polarization of THP-1 monocytes to proinflammatory macrophages

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ABSTRACT

The monocytes are key components of innate immunity, as they can differentiate into phagocytic cells or macrophages with proinflammatory or anti-inflammatory phenotypes. The gamma-aminobutyric acid (GABA) and adenosine triphosphate (ATP), two known neurotransmitters, are two environmental signals that contribute to the differentiation of monocytes into macrophages and their subsequent polarization into proinflammatory M1 and anti-inflammatory M2 macrophages. Although monocytes and macrophages express proteins related to GABA and ATP-mediated response (GABAergic and purinergic systems, respectively), it is unknown whether changes in their expression occur during monocyte activation or their differentiation and polarization into macrophages. Therefore, we evaluated the expression levels of GABAergic and purinergic signaling components in the THP-1 monocyte cell line and their changes during monocyte activation, differentiation, and polarization to M1 proinflammatory macrophages. Our results showed that activated monocytes are characterized by increased expression of two GABAergic components, the GABA transporter 2 (GAT-2) and the glutamic acid decarboxylase (GAD)-67, an enzyme involved in GABA synthesis. Also, monocytes showed a pronounced expression of the purinergic receptors P2X4 and P2X7. Interestingly, during differentiation, monocytes increased the expression of the β2 subunit of GABA A-type receptor (GABA-AR), while the purinergic receptors P2X1 and P2X1del were reduced. In contrast, proinflammatory M1 macrophages showed a reduced expression in the α4 subunit of GABA-AR and GAD67, while P2X4 and P2X7 were overexpressed. These results indicate that dynamical changes in the GABAergic and purinergic components occur during the transition from monocytes to macrophages. Since GABA and ATP are two neurotransmitters, our results suggest that monocytes and macrophages respond to neurotransmitter-induced stimulation and may represent a path of interaction between the nervous and immune systems during peripheral inflammation and neuroinflammation development.

1. Introduction

Monocytes are key cells in the innate immune response since they can differentiate into specialized phagocytic cells known as macrophages [1]. During embryogenesis, monocytes originate from progenitor cells in the yolk sac and then migrate to specific host tissues, where they mature into tissue-resident macrophages [2]. During tissue-resident macrophage renewal or in an inflammatory process, monocytes in the bloodstream will be recruited to the target tissue, where they differentiate into macrophages to phagocyte pathogens or cellular components

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and promote tissue repair and maintenance [2,3].

Monocyte differentiation into macrophages begins with inflammatory mediators such as cytokines, chemokines, and growth factors [3]. Subsequently, microenvironment stimuli will favor macrophage activation or polarization, providing them with specific functions and characteristics. For example, macrophage colony-stimulating factor (M-CSF) induces polarization to a non-activated M0 phenotype, which, followed by interleukin (IL)-4 stimulation, leads to an anti-inflammatory and proliferative M2 phenotypes [4]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) or lipopolysaccharides (LPS), a component of the cell membrane of bacteria, stimulates macrophage polarization to proinflammatory M1 phenotype [4,5].

Each macrophage phenotype has a particular set of surface markers and releases specific immune mediators. M0 macrophages highly express the macrophage-specific marker cluster of differentiation (CD)68 and lack pro- or anti-inflammatory activation protein markers [6,7]. M1 macrophages, on the other hand, overexpress proinflammatory cytokines and chemokines such as IL-1β and tumor necrosis factor (TNF)-α, among others [7]. M2 macrophages overexpress anti-inflammatory cytokines and chemokines such as IL-10, C–C motif ligand (CCL)18, and CCL22 [7]. Therefore, it is known that surface markers and the release of proinflammatory or anti-inflammatory mediators define macrophage phenotype. However, the mechanism that governs the transition from monocyte to macrophage is still not completely understood, partly due to the high complexity of the microenvironment-induced signaling mechanisms.

Research evidence indicates that neurotransmitters such as gamma-aminobutyric acid (GABA) and adenosine triphosphate (ATP) can activate or modulate immune cells' function [8,9]. GABA-producing B lymphocytes modulate monocyte-to-macrophage differentiation with an anti-inflammatory phenotype [9]. Alveolar macrophages express the GABA A-type receptor (GABA-AR) and glutamic acid decarboxylase (GAD) -an enzyme that participates in GABA synthesis, the expression of these two proteins contributes to the polarization of M2 macrophages [10]. Alongside GABA, extracellular ATP is a key component in monocyte differentiation by activating ionotropic purinergic receptors P2X1, P2X4, and P2X7 [8,11,12], but less is known about whether purinergic receptor splice variants such as P2X1del contribute to monocyte differentiation and activation in macrophages.

Monocytes and macrophages play a crucial role in various diseases, including autoimmune diseases, cancer, obesity, and neuroinflammation [5,13], and since GABA and ATP modulate these immune cells' function, we evaluated whether changes in the expression of GABA and ATP signaling components in THP-1 monocytes occur during monocytes activation, differentiation, and polarization to proinflammatory M1 macrophages.

2. Materials & methods

2.1. Cell culture and differentiation of THP-1 cells

THP-1 cells were incubated in RPMI-1640 medium (Gibco) supplemented with 10 % Fetal Bovine Serum (Gibco) and 1 % penicillin/streptomycin in a humidified incubator at 37 °C with 5 % CO_2; cells were seeded in 6-well plates at a concentration of $5x10^5$ cells in 5 mL. Four experimental conditions were evaluated. The control cells were kept for five days in the absence of stimuli. To evaluate THP-1 monocyte activation, after four days without any stimuli, cells were incubated with 10 ng/mL LPS for the remaining 24 h. To induce the differentiation of THP-1 monocytes to macrophages (M0), cells were incubated with 1 ng/mL phorbol-12-myristate-13-acetate (PMA) (Sigma-Aldrich) for the initial 72 h only. After this period, cells were kept in a fresh medium for 48 h. To induce monocytes' proinflammatory phenotype or M1 macrophages, cells were incubated with PMA (1 ng/mL) for 72 h. Then, the medium was replaced and cells were kept in a fresh medium for 24 h, then incubated with 10 ng/mL LPS (Sigma-Aldrich) during the last 24 h; this method was previously reported by [14]. After concluding each experimental condition, the cells were detached with 1 mM EDTA phosphate-buffered saline (PBS) solution for 15 min; each cell suspension was centrifuged at 1500 rpm for 5 min, and the cell pellet was kept for the following experimental evaluation. Cell viability was determined by trypan blue staining; viable samples were considered when ≥95 % of living cells were present.

2.2. Flow cytometry analysis

Each cell pellet was resuspended in PBS and cells were counted with a Neubauer device to add $5x10^5$ cells in individual tubes. To corroborate THP-1 cell differentiation to macrophages, we evaluated the content of CD14 surface labeling by flow cytometry; low levels of CD14 are expected in THP-1 monocytes, and an increase occurs during differentiation and polarization [14]. Cells were incubated with phycoerythrin (PE) anti-human CD14 antibody (BioLegend) for 20 min at 4 °C. Then a washing step was carried out with 1 mL of PBS, the supernatant was discarded, and the cells were resuspended in 200 μL of PBS to be immediately analyzed in a FACSCanto II flow cytometer (BD Biosciences™) and using FlowJo software (Becton, Dickinson and Company). To determine CD14 levels by flow cytometry, the THP-1 cell population was first analyzed based on its size (FSC-A) and granularity (SSC-A). Then, the singlets were selected, and the doublets were discarded with the analysis of FSC-H vs. FSC-A. Finally, the mean fluorescence intensity (MFI) from $CD14^+$ cells was evaluated.

2.3. RT-qPCR

Each cell pellet was lysed with TRIzol (Ambion) to obtain the total ribonucleic acid (RNA) using a phenol-chloroform gradient. Complementary deoxyribonucleic acid (cDNA) was synthesized from 1 μg of total RNA in 10 μL using the High-Capacity cDNA Reverse Transcription kit (Thermo Scientific). The cDNA concentration for all samples was evaluated and adjusted to 100 ng/μL. Real-time PCR was carried out on a CFX96 (Bio-rad) real-time system. The PCR reaction was performed in a total volume of 10 μL containing 3 μL of cDNA, 5 μL of Maxima SYBR Green Master Mix 2x (Thermo Scientific), 0.3 μL of each primer (100 mM), and 1.4 μL of H_20 DEPC. The sequences of the primers used in this study are shown in Table 1. We used a previously reported normalized expression method to obtain the relative expression of each gene of interest, using two endogenous genes (βActin and 18S rRNA) [15]. The basal expression levels of each mRNA in THP-1 monocytes were calculated using the ΔCT value, where the CT value inversely correlated to the amount of mRNA, so a lower ΔCT is a higher mRNA abundance. The expression profile is reported as the change in the percentage of expression.

2.3.1. Statistical analysis

Data were analyzed using the GraphPad Prism v.9 software (GraphPad Software Inc., San Diego, CA, USA). We used the Shapiro-Wilk test to determine the distribution of each variable. After confirming that our data followed a normal distribution, we used one-way ANOVA followed by a Tukey post hoc test to determine the differences between the experimental groups. To determine differences in the percentage of expression relative to the control group, we used one-way ANOVA followed by Dunnett's post hoc test. We used the Kruskal-Wallis test for non-parametric data, followed by Dunn's post hoc test. Data are expressed as the mean ± the standard error; a statistically significant difference was considered with a $p < 0.05$.

3. Results

3.1. Confirmation of THP-1 monocyte differentiation and polarization

To corroborate THP-1 monocytes differentiated into macrophages

Table 1
Primers used for RT-qPCR analysis.

Gene	Accession numbers	Primers (5′–3′)	Product lengths (bp)
ACTB	NM_001101.5	**FW:** CCCTGGAGAAGAGCTACG **RV:** TGAAGGTAGTTTCGTGGATG	135
RNA18S	NR_145820.1	**FW:** GGCCCTGTAATTGGAATGAGTC **RV:** CCAAGATCCAACTACGAGCTT	146
P2X1	NM_002558.4	**FW:** TTGTGGAGAACGGGACCAA **RV:** GTCAAAGCGAATCCCAAACAC	59
P2X1del	Isoform of NM_002558.4 [11]	**FW:** CCAACCACTCCACCCTTCTC **RV:** GACATCCCGCGCATCAGC	197
P2X4	NM_001256796.2	**FW:** GCCGCCTCGATACACGGGAC **RV:** TGCTCGTTGCCAGCCAGGTC	94
P2X7	NM_002562.6	**FW:** CGGCTCAACCCTCTCCTACT **RV:**GGAGTAAGTGTCGATGAGGAAGTC	67
NLRP3	NM_001243133.2	**FW:** GAAGTGGACTGCGAGAAGTT **RV:** CGTTCGTCCTTCCTTCCTTT	106
IL1B	NM_000576.3	**FW:** CTCTCACCTCTCCTACTCACTT **RV:** TCAGAATGTGGGAGCGAATG	95
GABRA4	NM_000809.4	**FW:** GGGTCCTGTTACAGAAGTGAAA **RV:** CTGCCTGAAGAACACATCCA	100
GABRB2	NM_021911.3	**FW:** CCTTCCATCCTGATTACCATCC **RV:** TGTGAGGACAGTTGTGATTCC	99
GAD67	NM_000817.3	**FW:** GGAACTGGCTGAATACCTCTATG **RV:** CCCTGAGGCTTTGTGGAATA	119
SLC6A13 (GAT-2)	NM_016615.5	**FW:** GCCAGTTTGTGTGTGTAGAAAG **RV:** CAAGGAAGGAGACGACAGATAC	120

(M0) or polarized to a proinflammatory phenotype (M1), we evaluated the changes in cells' size, granularity, and content of CD14. Fig. 1A shows an example of the flow cytometry analysis used. The geometric mean of FSC-A and SSC-A showed that after the differentiation treatment with PMA and polarization with PMA + LPS conditions, cells significantly increased in size compared to the control group (Fig. 1B). Although PMA and PMA + LPS increased the granularity of the cells, only the PMA + LPS led to a significant increase compared to the control group (Fig. 1C). Relative to the control condition, M0 macrophages (PMA) and M1 macrophages (PMA + LPS) showed a significant increase in $CD14^+$ compared to monocytes (Fig. 1D). Relative to PMA, PMA + LPS significantly reduced $CD14^+$ (Fig. 1D).

These results show that THP-1 cells increase in size and granularity during the differentiation of monocytes to macrophages and that THP-1 monocytes increase $CD14^+$ content during differentiation and polarization, which confirms their macrophage phenotype.

Next, we evaluated the mRNA expression levels of two genes related to a proinflammatory phenotype, the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) and IL-1β. Notably, a 50 % reduction in NLRP3 expression occurs in M0 and M1 macrophages compared to monocytes (Fig. 2A). In contrast, relative to monocytes, M1 macrophages increased nearly 200-fold in IL-1β expression (Fig. 2B).

Altogether, these results confirm that the PMA and LPS treatment effectively induced the differentiation of THP-1 monocytes to macrophages and their polarization to a proinflammatory phenotype.

3.2. Expression of GABAergic components and their expression profile from THP-1 monocytes to M1 macrophages

THP-1 monocytes express components of the GABAergic signaling [16]. Therefore, we evaluated expression levels of these two subunits of GABA-AR (GABA-ARα4 and GABA-ARβ2), the GABA transporter GAT-2, and the enzyme GAD67 in unstimulated THP-1 monocytes and during the monocyte activation, polarization, and differentiation processes (Supplementary Fig. 1). Relative to monocytes, only polarized M1 macrophages showed a pronounced reduction in the expression levels of the GABA-ARα4 subunit; (Fig. S1A). On the contrary, relative to the control group, M0 macrophages showed a 2-fold increase in expression levels of the GABA-ARβ2 subunit, while M1 macrophages showed no changes (Fig. S1B). Similar levels of GAT-2 expression were observed in all conditions evaluated (Fig. S1C). Compared to control monocytes, LPS-stimulated monocytes showed a 2-fold increase in GAD67. In contrast, relative to LPS-stimulated monocytes, M1 macrophages showed reduced expression of GAD67 (Fig. S1D).

Unstimulated THP-1 monocytes express all GABAergic components evaluated, where GABA-ARα4 and GABA-ARβ2 showed the highest expression relative to their housekeeping genes (Fig. 3A). Next, we analyzed the different GABAergic mRNAs' expression profiles in each condition relative to the control. Monocytes activated with LPS showed no change in GABA-AR subunits, while expression of GAT-2 and GAD67 increased (Fig. 3B). M0 macrophages only showed a marked increase in the expression of the GABA-ARβ2 subunit (Fig. 3C). In contrast, M1 macrophages showed markedly reduced expression of α4 subunit and GAD67 (Fig. 3D).

These results indicate that THP-1 monocytes express GABA-ARα4, GABA-ARβ2, GAT-2, and GAD67, whose expression changes along the different stages of monocyte activation. Activated THP-1 monocytes overexpress GAT-2 and GAD67, the highest expression of GABA-ARβ2 characterized non-activated macrophages, while M1 macrophages downregulated the GABA-ARα4 and GAD67 expression.

3.3. Expression levels of purinergic receptors from THP-1 monocytes to M1 macrophages

We determined the transcriptional expression levels of P2X1, P2X1del isoform, P2X4, and P2X7 receptors under activation, differentiation, and polarization conditions in THP-1 cells (Supplementary Fig. S2). Relative to monocytes, P2X1 purinergic receptors and its isoform P2X1del showed a 50 % decrease in M0 macrophages, an effect that is more pronounced in M1 macrophages (Figs. S2A and S2B). LPS-stimulated monocytes and M1 macrophages significantly increased P2X4 receptor expression (Fig. S2C). The increased expression of P2X4 in M1 also differed from the levels detected in M0 macrophages (Fig. S2C). Likewise, LPS-stimulated monocytes showed a significant increase in the expression levels of the P2X7 receptors (Fig. S2D). Relative to expression levels in M0, M1 macrophages showed a 2-fold increase in P2X7 expression (Fig. S2D).

Next, we evaluated the expression profile of P2X receptors during the transition from an unstimulated monocyte to an M1 macrophage. We observed that unstimulated THP-1 monocytes express the different P2X receptors, where P2X1 and P2X4 showed the highest expression relative

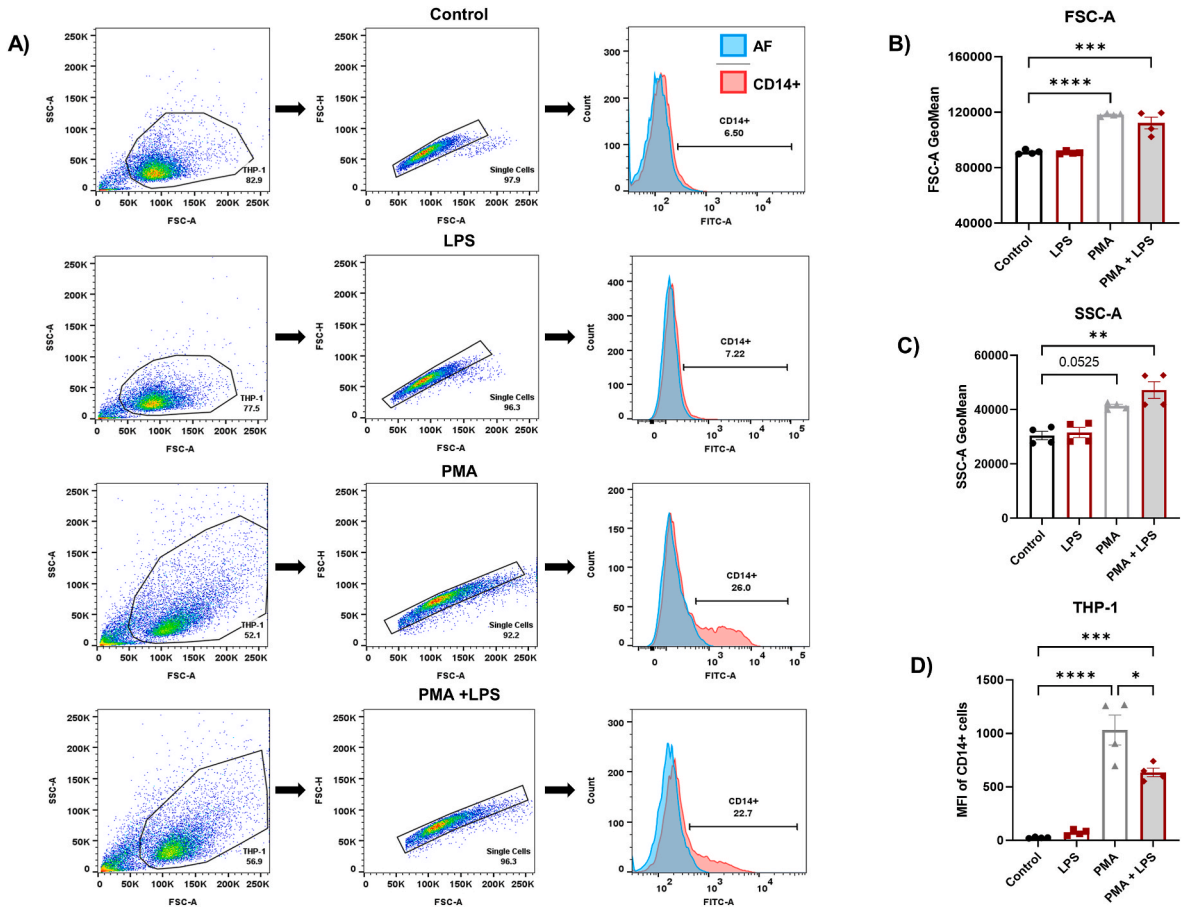

Fig. 1. Evaluation of differentiation and polarization markers by flow cytometry. An example of the analysis used to determine cell size (FSC-A), granularity (SSC-A) and CD14 content by flow cytometry in each experimental condition (A). First, cell size and granularity were considered. Next, the singlets were selected and the mean fluorescence intensity (MFI) from $CD14^+$ cells was evaluated using an unstained control to remove the autofluorescence (AF) signal. The geometric mean of FSC-A indicated changes in cell size in each condition (B). The geometric mean of SSC-A indicated changes in cell granularity (C). MFI levels of $CD14^+$ cells in activation, differentiation, and polarization stages (D). Each data point consists of four independent experiments performed by triplicates. Data were analyzed using a one-way ANOVA and Tukey's post hoc analysis or Kruskal-Wallis and Dunn's post hoc tests. The results are shown as the mean ± SEM, a $p < 0.05$ was considered significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

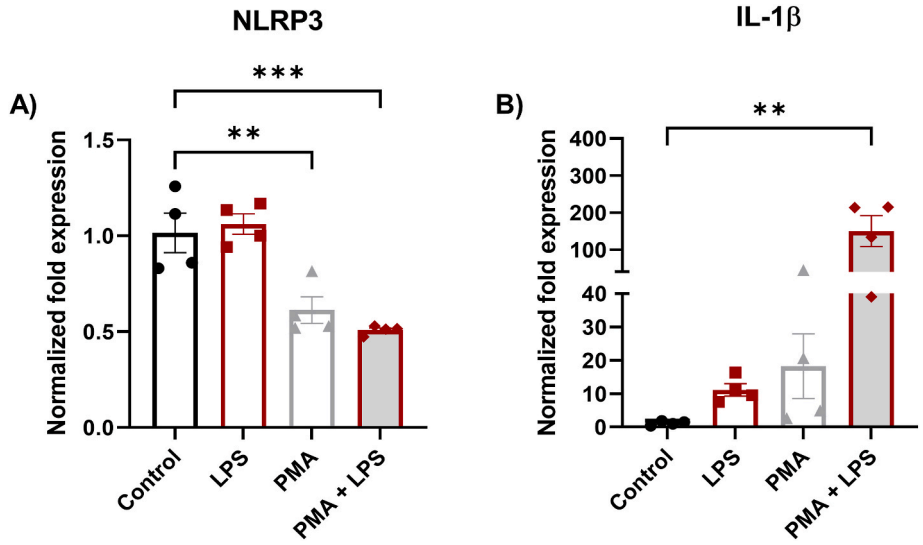

Fig. 2. Expression levels of proinflammatory markers by RT-qPCR. Graphs show the normalized expression for NLRP3 (A) and IL-1β (B) from THP-1 monocytes and during their activation, differentiation, and polarization. Each determination was performed in four independent experiments by triplicates. Data were analyzed using a one-way ANOVA and Tukey's post hoc analysis. The results are shown as the mean ± SEM, a $p < 0.05$ was considered significant. **p<0.01, ***p<0.001.

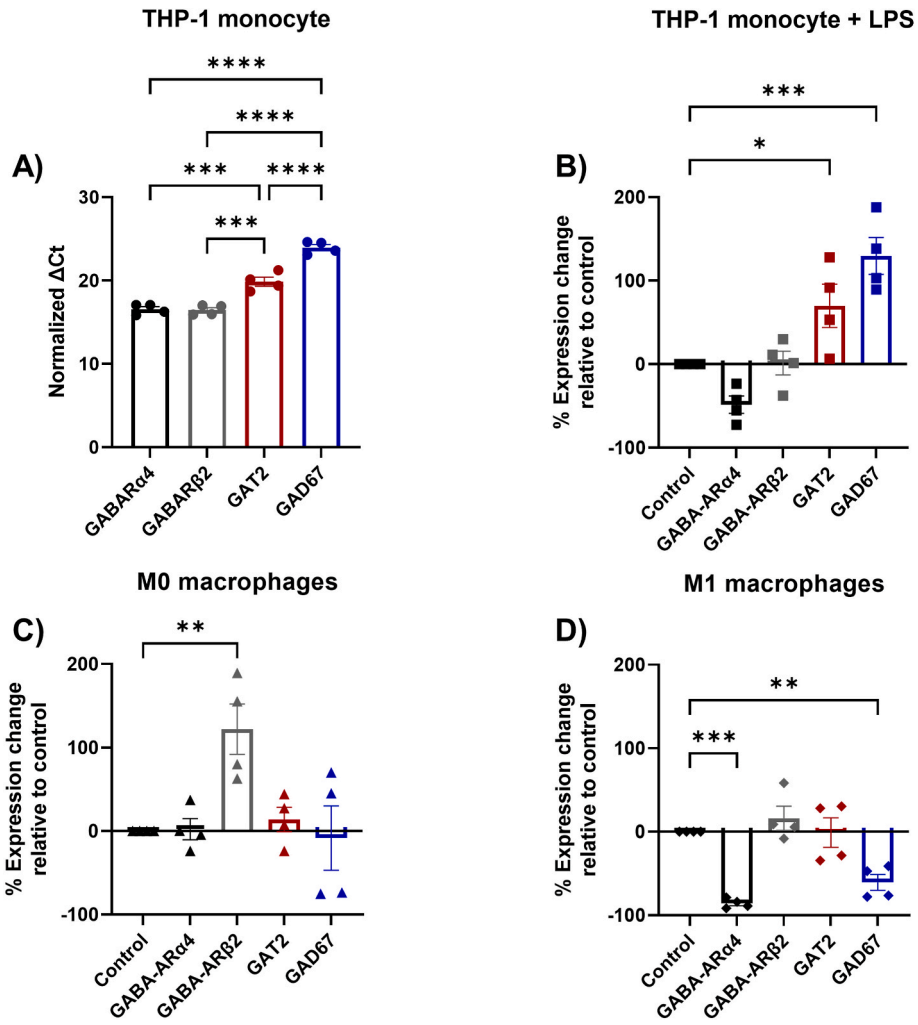

Fig. 3. Changes in the expression profile of GABAergic components in unstimulated THP-1 cells and under activation, differentiation, and polarization conditions. The graphs show the basal genes expression as the normalized ΔCT value (A) and the percentage of change in the expression of the α4 and β2 subunit of the GABA-A receptor, the GABA transporter type 2, and the glutamic acid decarboxylase of 67 KDa in unstimulated THP-1 monocytes (Control) compared to THP-1 monocytes stimulated with LPS (B), M0 macrophages (C) or M1 macrophages (D). The different determinations were performed in four independent experiments by triplicates and the data were analyzed using a one-way ANOVA and Dunnett or Tukey post hoc analysis. The results are shown as the mean ± SEM, a $p < 0.05$ was considered significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

to their housekeeping genes (Fig. 4A). Monocytes stimulated with LPS are characterized by a marked increased expression of P2X4 and P2X7 receptors (Fig. 4B). Although M0 macrophages showed an important reduction in P2X1 and P2X1del, only P2X1del reduction was statistically significant (Fig. 4C). Finally, M1 macrophages showed a prominent increase in the expression of the P2X4 receptors (Fig. 4D).

These results suggest that while P2X1 and P2X1del receptors are downregulated during differentiation of monocyte-to-macrophage, P2X4 and P2X7 are overexpressed under pro-inflammatory conditions. Therefore, changes in the expression profile of purinergic receptor underlie the transition from monocyte to macrophage activation, differentiation, and polarization.

4. Discussion

Depending on the surrounding microenvironment, monocyte-derived macrophages can exhibit either an inflammatory or anti-inflammatory phenotype [17]. However, given the variable nature of the microenvironment, the monocyte differentiation processes to macrophages and their subsequent polarization toward a specific phenotype are highly complex. To better understand this process, we evaluated key components of GABAergic and purinergic signaling in the THP-1 monocyte cell line during activation, differentiation, and polarization stages toward M1 macrophages. Our results suggest that activating THP-1 monocytes with LPS increased the expression of GAT-2, GAD67, P2X4 and P2X7; a different phenotype from that observed in M0 macrophages where GABA-ARβ2 is overexpressed and P2X1del is downregulated, and M1 macrophages under-express GABA-ARα4 and GAD67 and markedly increase the P2X4 expression. Therefore, THP-1 monocytes and THP-1-derived macrophages express GAT-2, GAD67, and P2X1del, and changes in these genes occur during the transition from monocytes to macrophages. This is the first study that reports changes in GABAergic and purinergic signaling components occur during the transition from monocyte to M1 macrophage using the THP-1 cells.

Monocyte differentiation and polarization increase cell size and granularity due to changes in macrophage morphology and an increased number of lysosome and mitochondria [14,18]. Also, THP-1 derivate--macrophages showed a larger expression of CD14, a protein commonly used as a macrophage marker [19], which is reduced under polarization conditions, a dynamic associated with the activation of M0 differentiation and M1 polarization process [14]. Therefore, these results confirm the induction of differentiation and polarization of THP-1 monocytes.

THP-1 cells stimulated with PMA and LPS (M1) increased the IL-1β expression, suggesting a greater production of proinflammatory

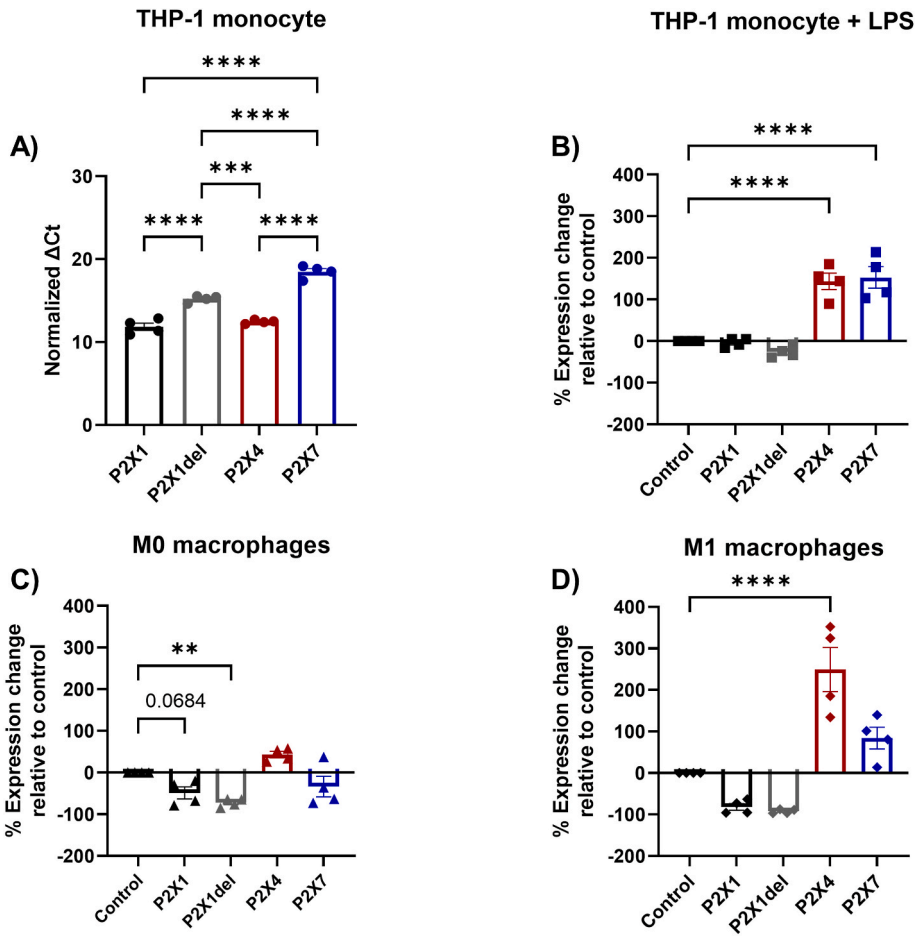

Fig. 4. Expression profile of purinergic components in unstimulated THP-1 cells and under activation, differentiation, and polarization conditions. Basal expression levels of P2X receptors in unstimulated THP-1 cells using the normalized ΔCT value (A). Percentage changes in the expression profile of P2X receptors in unstimulated THP-1 monocytes (Control) compared to THP-1 monocytes stimulated with LPS (B), M0 macrophages (C) or M1 macrophages (D). The different determinations were performed in four independent experiments by triplicates and the data were analyzed using a one-way ANOVA and Dunnett or Tukey post hoc analysis. The results are shown as the mean ± SEM, a $p < 0.05$ was considered significant. **p<0.01, ***p<0.001, ****p<0.0001.

cytokines in this phenotype. Although increased NLRP3 expression is associated with IL-1β maturation and release, our results showed low NLRP3 expression, suggesting an NLPR3-independent mechanism of IL-1β maturation in THP-1 macrophages. However, the protein content of NLRP3 and IL-1β mRNA should be corroborated.

GABA is an inhibitory neurotransmitter, but recent reports indicate it also activates or modulates cellular components of the immune response. Thus, GABA might be central to the communication between the nervous and the peripheral immune systems [9,10,20,21]. Monocytes and macrophages express mainly GABA-AR, composed of two α, two β, and one γ subunits [22]. Our data showed a major presence of GABA-AR in M0 macrophages, with an increased β2 subunit expression; this subunit is needed for the inhibitory activity of the GABA-AR [23]. However, direct evaluation of the function of these receptors in THP-1 monocytes and macrophages is warranted.

Since GABA promotes the differentiation of monocytes into anti-inflammatory macrophages [9], changes in GABA-AR expression might underlie macrophage polarization. Given that M0 macrophage exhibits M2-like properties related to an anti-inflammatory phenotype, the increase in GABA-AR could be associated with this phenotype [4]. A previous study showed increased expression of α4 and β2 subunits in M0 macrophages derived from THP-1 monocytes [16]. In contrast, M1 macrophages showed a reduction in α4 and β2 expression, suggesting that polarization of macrophages to the M1 phenotype requires a reduced GABA-AR, especially those that express the α4 subunit, a mechanism that would prevent the anti-inflammatory activity of GABA.

In this regard, commonly used anesthetics such as propofol, activate GABA receptors in the brain. However, this anesthetic has been shown to increase the expression of GABA receptors α4 and β2 subunits during monocyte polarization to M1 macrophage *in vitro* [16]. Interestingly, propofol also reduces the production of proinflammatory cytokines such as IL-6 or IL-1β in M1 macrophages [24]. Therefore, using GABA receptor agonists for anesthetizing purposes might inhibit the activation and polarization of M1 macrophages or other immune cells expressing GABA receptors, which might hamper immune response during infection conditions.

GAT-2 is a sodium- and chloride-dependent GABA transporter expressed in peripheral tissues and the brain where it regulates extracellular GABA concentrations through its reuptake [25]. Our results showed that THP-1 monocytes activated with LPS increased GAT-2 expression. Interestingly, macrophages from brain samples of patients with multiple sclerosis showed increased GAT-2 expression [26]. In the immune system, GAT-2 expression levels modulate T lymphocytes and macrophages' proinflammatory activity [27,28]. Therefore, increased GAT-2 expression suggests an increase in GABA uptake, which could reduce the extracellular concentration of GABA and then shape THP-1 monocyte activation. We observed changes in GABAergic receptors and transporter expression during the transition from monocytes to macrophages. An interesting question that emerges is if the production of GABA changes too. Therefore, we evaluated the expression of GAD67, an enzyme involved in GABA synthesis [29]. Monocytes activated with LPS increased the expression of GAD67, and M1 macrophages showed a

reduction in the expression of GAD67, suggesting that activated monocytes synthesize GABA. To our knowledge, this is the first report describing that THP-1 monocytes express GAD67 during activation, differentiation, and polarization.

Although extracellular levels of GABA need to be evaluated, these results open the question about the mechanisms of GABA release in THP-1 monocytes and macrophages. To date, three main mechanisms have been described, the vesicle-related release, GABA-permeable anion channels, and the reverse function of the transporters [30,31], and these mechanisms should be evaluated in THP-1 cells.

Interestingly, dendritic cells (phagocytic and antigen-presenting cells) under infectious challenge express GAD67 and its inhibition reduces the cell motility, a mechanism required to induce the adaptive immune response [32]. This suggests that GABA synthesis and release in activated monocytes could stimulate cell motility in an autocrine way, a mechanism required for these cells to migrate and reach the target tissue.

The P2X1 and P2X1del expression was reduced under differentiation (PMA) and polarization (PMA + LPS) conditions, where the M0 macrophages show a clear reduction of P2X1del. Therefore, THP-1 monocyte differentiation and polarization downregulate P2X1 and P2X1del, an effect previously described only under differentiation conditions in human monocytes and monocyte-derived macrophages [12,33,34].

LPS-activated THP-1 monocytes and M1 macrophages showed increased P2X4 and P2X7 expression. Previous reports described that THP-1 monocytes and macrophages mainly express P2X4 under non-activation conditions, which leads to calcium influx [33]. Our findings suggest that THP-1 monocyte activation leads to co-expression of P2X4 and P2X7, which under inflammatory conditions characterized by high concentrations of extracellular ATP (mM range), could induce the production of proinflammatory cytokines as IL-1β. Furthermore, since the P2X4 receptor enhances P2X7-mediated inflammation, the mainly increased expression of P2X4 in M1 macrophages could be necessary to modulate the P2X7-dependent release of IL-1β. However, studies evaluating the activation, differentiation, and polarization of THP-1 monocytes in the presence of ATP and selective inhibitors of purinergic receptors are necessary to understand the role of purinergic receptors under each condition.

In conclusion, our results indicate that THP-1 monocytes transition to M1 macrophages involves a dynamic change in the expression of GABA receptor subunits, the enzyme synthesizing GABA, and its transporter GAT-2, along with changes in P2X1, P2X1del, P2X4, and P2X7. This evidence indicates a possible key role of GABA and ATP as modulators of the peripheral immune response. Moreover, since GABA and ATP are two neurotransmitters, activating these signaling pathways in monocytes and macrophages might represent direct communication between the nervous and immune systems. Finally, GABAergic components in monocytes and macrophages might represent a potential therapeutic target for macrophage-related diseases.

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Author statement

Victor Manuel Ruiz-Rodríguez: Conceived the idea presented. Conceived and planned the experiments. Participated in all the experiments. Directed the writing of the manuscript. Supervised the results of this work. **Carlos Alberto Torres-González**: Contributed to the culture of THP-1 cells and the preparation of samples for acquisition in the flow cytometer. **Karina Monserrat Salas-Canedo**: Contributed to THP-1 cell culture, RNA extraction and the evaluation of gene expression by RT-qPCR. **Nicole Quibey Pecina-Maza**: Contributed to THP-1 cell culture and evaluation of gene expression by RT-qPCR. **Miguel Ernesto Martinez-Leija**: Contributed to the development of RT-qPCR. **Diana Patricia Portales-Pérez**: Contributed to the infrastructure for the development of the experiments and supervised the results of this work. **Ana María Estrada-Sánchez**: Conceived the idea presented. Conceived and planned the experiments. Provided funding for the development of the work. Supervised the results of this work. Directed the writing of the manuscript. All authors discussed the results and provided critical comments on the final manuscript.

Declaration of competing interest

All the authors signing the manuscript **"Dynamical changes in the expression of GABAergic and purinergic components occur during the polarization of THP-1 monocytes to proinflammatory macrophages"** submitted for publication to Biochemistry and Biophysics Reports declare no financial or personal relationship with other organizations or people that could influence this report.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101558.

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