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Original Article

Antimicrobial peptide LL-37 along with peptidoglycan drive monocyte polarization toward CD14^{high}CD16^{+} subset and may play a crucial role in the pathogenesis of psoriasis guttata

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Abstract: The human cathelicidin LL-37 peptide is overexpressed in psoriasis and has been demonstrated to be a multifunctional modulator of innate immune response elements, including monocytes. Monocytes, categorized into three populations based on the cell surface expression of CD14 and CD16, are activated in psoriasis guttate and are commonly triggered by streptococcal infections. Peptidoglycan (PGN) is a major cell-wall component of streptococcus, and an increasing number of PGN-containing cells have been detected in psoriasis. Since there are independent reports of both PGN and LL-37 influencing monocytes, we tried to evaluate the effect of human LL-37 on PGN-induced monocyte activity and differentiation and subsequently studied their correlation with the pathogenesis of psoriasis guttate. The results revealed that monocytes from the peripheral blood of healthy individuals resulted in their polarization toward the CD14^{high}CD16^{+} subset, when cultured with PGN in the presence of the LL-37 peptide. This peptide further induced PGN-driven differentiated monocytes into immature dendritic cells (iDC), as evident by the increased expression of CD1a, CD86, and HLA-DR markers, resulting in the induction of T cell proliferation and Th17 polarization. Furthermore, our data suggested that psoriasis guttata patients have significantly higher percentages of CD14^{high}CD16^{+} monocytes as well as circulating levels of LL-37, soluble form of triggering receptor expressed on myeloid cells (sTREM-1) levels, and anti-streptolysin O (ASO) levels, as compared to healthy controls. Psoriasis guttata patients also showed a positive correlation between the percentage of CD14^{high}CD16^{+} monocytes and the serum levels of sTREM-1 as well as the Psoriasis Area and Severity Index (PASI) scores. Therefore, we concluded that LL-37 in synergy with PGN directs monocyte polarization and differentiation into a proinflammatory phenotype, which might play a crucial role in the pathogenesis of psoriasis.

Keywords: Triggering receptor expressed on myeloid cells-1, psoriasis guttata, antimicrobial peptide LL-37, peptidoglycan, monocytes

Introduction

Psoriasis is a chronic, systemic, inflammatory, and multigenic disease characterized by red and scaly skin plaques. Its pathogenesis is characterized by aberrant keratinocyte proliferation and differentiation, development of new blood vessels, and infiltration of T lymphocytes, dendritic cells (DCs), neutrophils, and other elements of innate immunity [1]. Psoriasis is basically classified into five morphological subtypes [2], but it has been reported that some phenotypes of this disease can also transform into other clinical forms [3]. Among the five morphological subtypes of psoriasis, the guttate type is mostly linked to streptococcal infections and the Th17 immune response [4]. Recently, it has been shown that the microbiota in guttate psoriasis activate monocytes and play a crucial role in its pathogenesis [5, 6].

Based on the expression of the lipopolysaccharide receptor CD14 and the Fcγ-III receptor CD16, the monocytes in human peripheral

blood are classified into three distinct subpopulations: classical ($CD14^{high}CD16^{-}$), intermediate ($CD14^{high}CD16^{+}$), and non classical ($CD14^{low}CD16^{+}$) [7]. Emerging evidence indicates that each subpopulation has a unique gene signature and may have differential functions in inflammation and immunity [8]. Intermediate monocyte populations clearly show a higher expression pattern of TNFR1, TNFR2, HLA-DR, CCR2, and CCR5 proteins than classical and nonclassical monocytes. Thus, this subset would especially be more sensitive to cytokine and chemokine signals [9, 10].

Peptidoglycan (PGN), an important etiological factor for psoriasis, is a major cell-wall component of Gram-positive bacteria. It is not only essential for bacterial survival but is also a target for the innate immune response. The genes for PGN recognition proteins (PGRP-3 and PGRP-4) are located on chromosome 1q21, which is also a known locus for psoriasis, the PSORS4 gene [11]. PGN has been detected within antigen-presenting cells in psoriasis, and T cells isolated from psoriatic skin respond in an antigen-specific manner to streptococcal and staphylococcal PGN [12]. In addition, multiple independent studies have indicated that numerous bacteria reside in the dermis and dermal adipose tissue of normal and psoriatic skin. The genera staphylococcus and streptococcus may be involved in the psoriatic process [13, 14]. In addition, it has been proposed that bacteria in the tonsils, intestine, and throat may be responsible for initiating and maintaining psoriasis [15].

The cathelicidin LL-37, a small peptide of 37 amino acids, is overexpressed particularly in the inflamed skin in psoriasis. It is produced by resident skin cells such as keratinocytes, sebocytes, eccrine gland cells, and infiltrating immune cells [16, 17]. The serum LL-37 levels are elevated in patients with psoriasis compared with healthy controls [18]. LL-37 is best known for its integral role in killing pathogenic microorganisms and building a chemical shield around the skin [19]. However, LL-37 also has been shown to be capable of modifying host immune and growth responses, including pro- and anti-inflammatory activities [20, 21], promotion of chemotaxis, angiogenesis, and enhancing wound repair. It has diverse effects on cellular responses to different pattern recognition receptor ligands by a variety of mechanisms, depending on the cell type [22]. LL-37 has a strong anti-endotoxin function through toll-like receptor (TLR) 4 signaling [23] and can attenuate tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 production in macrophages by inhibiting lipoteichoic acid-induced p38MAPK and AKT phosphorylation [24]. In psoriasis, it has been suggested that LL-37 binds to extracellular self-DNAs released from dying cells and converts these self-DNAs into a potent stimulus for plasmacytoid dendritic cells. Subsequently, these plasmacytoid dendritic cells trigger an auto-inflammatory cascade through type I interferon secretion. Similarly, LL-37 can also transform self-RNAs into a potent stimulus for activation of myeloid dendritic cells through TLR7 and TLR8 signaling pathways [25].

Based on the current information, the consequences of continuous exposure of peripheral blood monocytes to PGN in the presence of LL-37 are not completely understood. Therefore, in this study, we assessed the effects of these molecules on the phenotype and function of peripheral blood monocytes *in vitro*. Since monocytes can also differentiate into immature DCs (iDCs), we also investigated the roles of LL-37 and PGN on monocytic differentiation towards iDCs. In addition, we assessed the ability of these resulting iDCs to function as antigen-presenting cells in a mixed lymphocyte reaction (MLR). Finally, we analyzed the monocyte subpopulations from psoriasis guttata patients and determined their correlation with different pathological characteristics in order to find new avenues for treatment of psoriasis.

Materials and methods

Study population

This study was approved by the Bioethics Committee of Peking Hepingli Hospital (EC/2014/002), and written informed consent was obtained from all participants. In our study, we enrolled 28 patients who were diagnosed with psoriasis guttata (16 women and 12 men; median age of 33.5 years old, range: 18-51 years old) and 24 age-matched healthy controls (13 women and 11 men; median age of 39 years old, range, 20-48 years old). The psoriasis diagnosis was based on clinical examination by dermatologists from the Department of Dermatology, Peking Hepingli Hospital. The

severity of the disease was assessed according to the Psoriasis Area and Severity Index (PASI).

Preparation of primary monocytes from healthy donors and their stimulation

The blood samples collected from healthy donors in sterile heparinized tubes were diluted with an equal volume of phosphate-buffered saline (PBS), pH 7.4 and centrifuged over Ficoll gradients (Amersham Pharmacia) to isolate peripheral blood mononuclear cells (PBMCs). The resulting PBMCs were further passed over Percoll gradients (Amersham Pharmacia) at a concentration of 5×10^7 cells/mL to deplete lymphocytes. The resulting primary monocytes were relatively pure (80-90%) as determined by $CD14^+$ staining and flow cytometry. Finally, the purified monocytes were cultured in complete RPMI 1640 medium (2 mM L-glutamine and penicillin- streptomycin) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco, Life Technologies) at 37°C in a 5% CO_2 incubator.

For activation, primary monocytes (1×10^5 cells/mL) were cultured in 24- and 96-well flat-bottom tissue culture plates (Corning) in the presence of 20 μg/mL LL-37 (InvivoGen) and 10 μg/mL PGN (InvivoGen) alone or together in complete media. After culturing for 16 h, the cell-free supernatants were analyzed for levels of IL-6, TNF-α, IL-1β, IL-8, IL-23, and granulocyte-macrophage colony-stimulating factor (GM-CSF), as described in the following section. The levels of triggering receptor expressed on myeloid cells (TREM-1), CD14, and CD16 on these cells were analyzed by flow cytometry. In addition, some purified monocytes were also cultured in the presence of 800 U/mL GM-CSF (Immunex) and 1000 U/mL IL-4 (Peprotech) for 5 days to generate iDC as described previously [26]. These iDCs were characterized by analyzing the expression of HLA-DR, CD1a, and CD86 markers by fluorescence-activated cell sorting (FACS) after 5 days of culture.

MLR and T cell assays

MLRs were performed to assess the ability of iDCs to elicit T cell proliferation and Th17 polarization, as described previously [27]. Briefly, iDCs were isolated, irradiated (3000 rad from a ^{137}Cs source), and cultured (1×10^4 cells/well) in 96-well plates. Allogeneic T cells purified from an unrelated donor using a T cell enrichment kit (Stem Cell Technologies) were allowed to adhere in RPMI 1640 media containing 1% FCS for 2 h to eliminate contaminating monocytes. The resulting T cells were determined to be 95% pure by flow cytometry. These T cells were added to the wells containing iDCs at a ratio of 1:20 (iDCs/T cells) and cultured for an additional 3 days. Thymidine incorporation was measured on day 3 after an 18-h pulse with [^{3}H] thymidine (1 μCi/well) by using standard procedures [28].

Detection of monocyte subpopulations from psoriasis guttata patients

Fresh heparinized blood samples from psoriasis guttata patients and healthy controls were collected. Peripheral blood samples (100 μL) were incubated with monoclonal FITC-labeled CD14 antibody (10 μL, BD Bioscience), PerCP-Cy5.5-labeled CD16 antibody (10 μL, BD Bioscience), and a phycoerythrin (PE)-labeled TREM-1 antibody (10 μL, R&D Systems) for 30 min in the dark. Subsequently, the red blood cells were lysed using BD FACS™ Lysing Solution (BD Biosciences) for 5 min. The cells were then washed twice with 1% bovine serum albumin in PBS. All steps were performed at room temperature. Flow cytometric analyses were done with a FACScan (BD Biosciences) flow cytometer and Cell-Quest software.

Cell-surface molecule expression analysis by FACS

Cells cultured in the presence of various stimuli were harvested and blocked with 20% human serum in 1 × PBS for 1 h on ice to reduce nonspecific antibody binding by FcRs. The cells were then stained with monoclonal PE-conjugated anti-CD86 (BD Bioscience), antigen-presenting cell (APC)-conjugated anti-HLA-DR (BD Bioscience), and PE-conjugated anti-CD1a (BD Bioscience), according to the manufacturer's protocols. For intracellular IL-17 cytokine staining, cocultured cells were incubated with 50 ng/mL Phorbol-12-Myristate-13-Acetate (PMA), 1 μg/mL ionomycin, and 1 μg/mL brefeldin (all from Sigma) for 4 h. T cells were labeled using PerCP-Cy5.5-labeled anti-CD4 antibodies. Next, the cells were fixed and labeled intracellularly using Intrastain reagents with PE-labeled antibodies directed against IL-17 (all from BD Biosciences). Flow cytometric

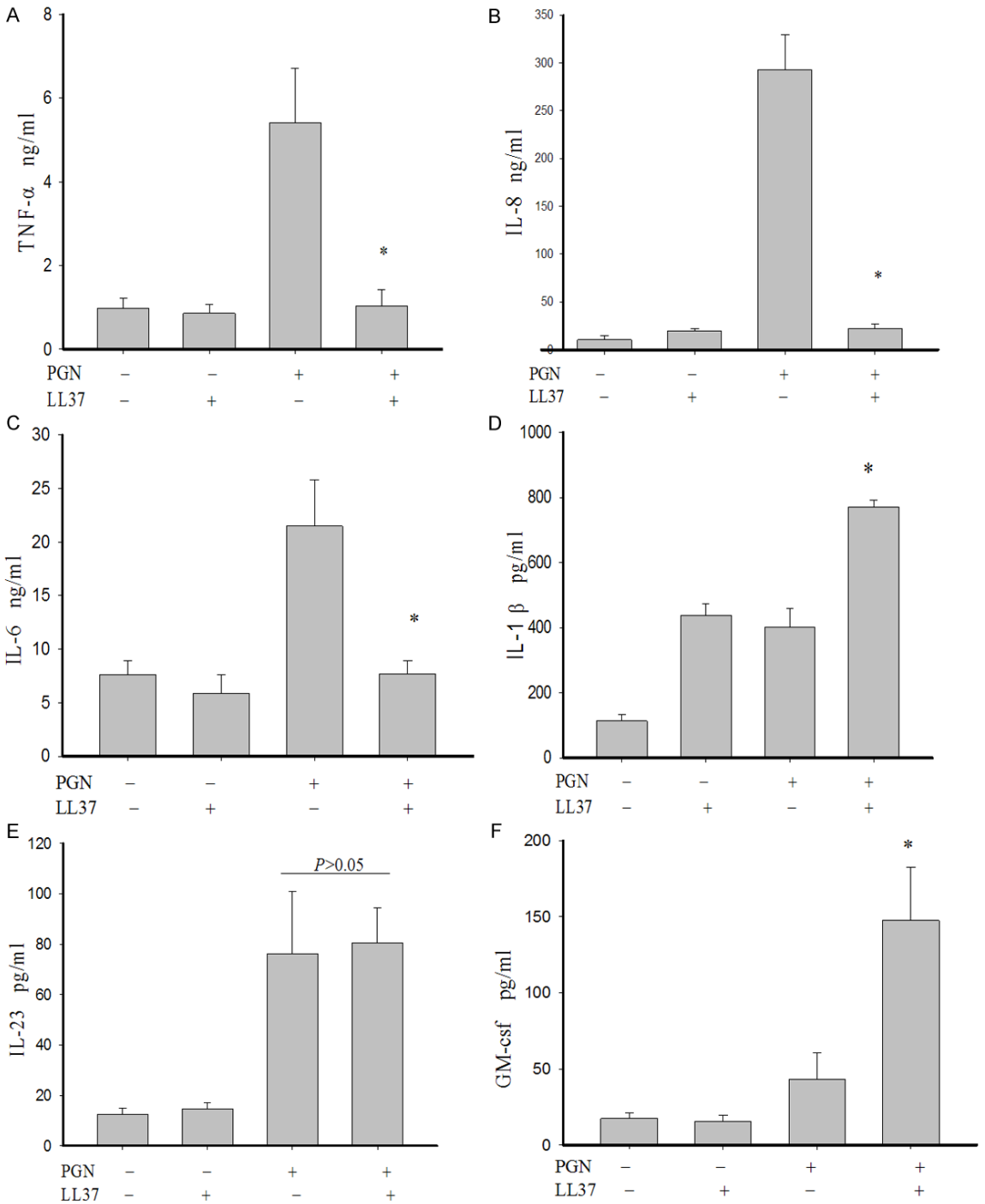

Figure 1. LL-37 affects PGN-induced cytokine production. Isolated monocytes (1×10^5 cells/mL) were treated with RPMI 1640 media (control), LL-37 peptide (20 μg/mL), PGN (10 μg/mL), or PGN + LL-37. Supernatants were collected after 16 h of treatment, and cytokine (TNF-α, IL-8, IL-6, IL-1β, IL-23, and GM-CSF) production was measured by the Luminex platform (panels A-F). Bars represent mean values ± standard errors of the mean (SEM). The asterisk represents a statistically significant difference compared with PGN-induced cytokine release ($P < 0.05$, n = 10). The statistical significance was determined by using the one-way ANOVA test.

analysis was performed using a FACS Calibur low laser cytometer (Becton Dickinson, USA). A minimum of 10,000 cells were analyzed for every sample.

Cytokine assay

Cell-free supernatants were analyzed for the production of IL-6, TNF-α, IL-1β, IL-8, IL-23, and GM-CSF by Luminex 200 platforms, according to the manufacturer's directions. Measurement of soluble TREM-1 (R&D Systems) and serum cathelicidin LL-37 (Hycult Biotech) was performed in duplicate by an enzyme-linked immunosorbnent assay with commercially available kits. The minimum detectable concentrations of serum LL-37 and soluble TREM-1 (sTREM-1) in this assay were 0.15 ng/mL and 15.1 pg/mL, respectively.

Statistical analysis

The Shapiro-Wilk's test was used to determine the normal distribution of quantitative variables. Parametric data were represented as the mean ± standard error of the mean (SEM). The differences between groups were analyzed by the T-test and one-way analysis of variance (ANOVA). Statistical significance between nonparametric data was determined by the Mann-Whitney U-test. Correlations between quantitative variables were analyzed with Pearson's correlation coefficient. $P < 0.05$ represented a statistically significant difference. All tests were two-sided. Analysis was performed with the STATA 7.0 statistical software package (Stata Corp., College Station, TX, USA).

Results

LL-37 modulates PGN-induced inflammatory monocytes

To investigate the role of LL-37 in modulating PGN-induced inflammatory monocytes, we treated these monocytes with 10 μg/mL PGN, 20 μg/mL LL-37, or their combination for 16 h. Analysis of the important inflammatory cytokines such as TNF-α, IL-8, IL-1β, GM-CSF, and IL-6 (**Figure 1A-F**) revealed that the PGN treatment alone significantly increased the inflammatory cytokines TNF-α, IL-8, IL-6, IL-1β, and IL-23, compared to the control treatment. In contrast, LL-37 treatment alone had no effect on TNF-α, IL-8, or IL-6 concentrations, compared to the control group; but the level of inflammatory cytokine IL-1β was increased significantly. Furthermore, the addition of LL-37 to PGN treatment resulted in inhibition of the PGN-induced increase of TNF-α, IL-8, and IL-6 levels. Meanwhile, this combined treatment had a synergistic effect on IL-1β and GM-CSF as their levels were increased significantly. However, IL-23 expression was not altered by the addition of LL-37 to PGN. Therefore, these data overall suggest that exogenous addition of chemically synthesized LL-37 could modulate PGN-induced inflammatory monocytes.

LL-37 along with PGN induces monocyte polarization

To elicit the roles of LL-37 and PGN in monocyte polarization, we incubated monocytes with PGN or LL-37 peptide alone or in combination for 16 h. Thereafter, we analyzed the expression of the cell-surface molecules TLR2, TREM-1, HLA-DR, CD16, and CD14 by flow cytometry. The data (**Figure 2A-E**) revealed that the levels of TREM-1 protein on monocytes were upregulated by PGN stimulation, and this effect was further enhanced by LL-37 as reported previously [29]; however, LL-37 alone had no effect on its levels. Neither PGN nor LL-37 alone affected the cell surface expression of HLA-DR or TLR2 on monocytes; but, surprisingly, LL-37 together with PGN reduced TLR2 expression and, conversely, enhanced HLA-DR expression. Also, LL-37 and PGN together resulted in an increase of the $CD14^{high}CD16^{+}$ subset of monocytes. Thus, these results suggested that LL-37 and PGN synergistically induce monocyte polarization.

LL-37 and PGN synergistically trigger primary monocyte differentiation into iDCs

Based on our observation of the increased TERM-1 expression and induction of GM-CSF by LL-37 and PGN, we explored their roles in primary monocyte differentiation into iDCs because TERM-1 plays a central role in monocyte differentiation into iDCs [30] and the cytokine GM-CSF has been shown to be essential for the generation of iDCs from monocytes *in vitro* [31]. Therefore, to further investigate this possibility, monocytes were cultured with PGN in the presence of either LL-37 or no peptide for 5 days to allow differentiation. Subsequent analysis of these cells for monocyte- and DC-specific markers by flow cytometry (**Figure 3A**) revealed that the combination of LL-37 and PGN increased CD1a expression concomitant with a downregulation of CD14. This represents

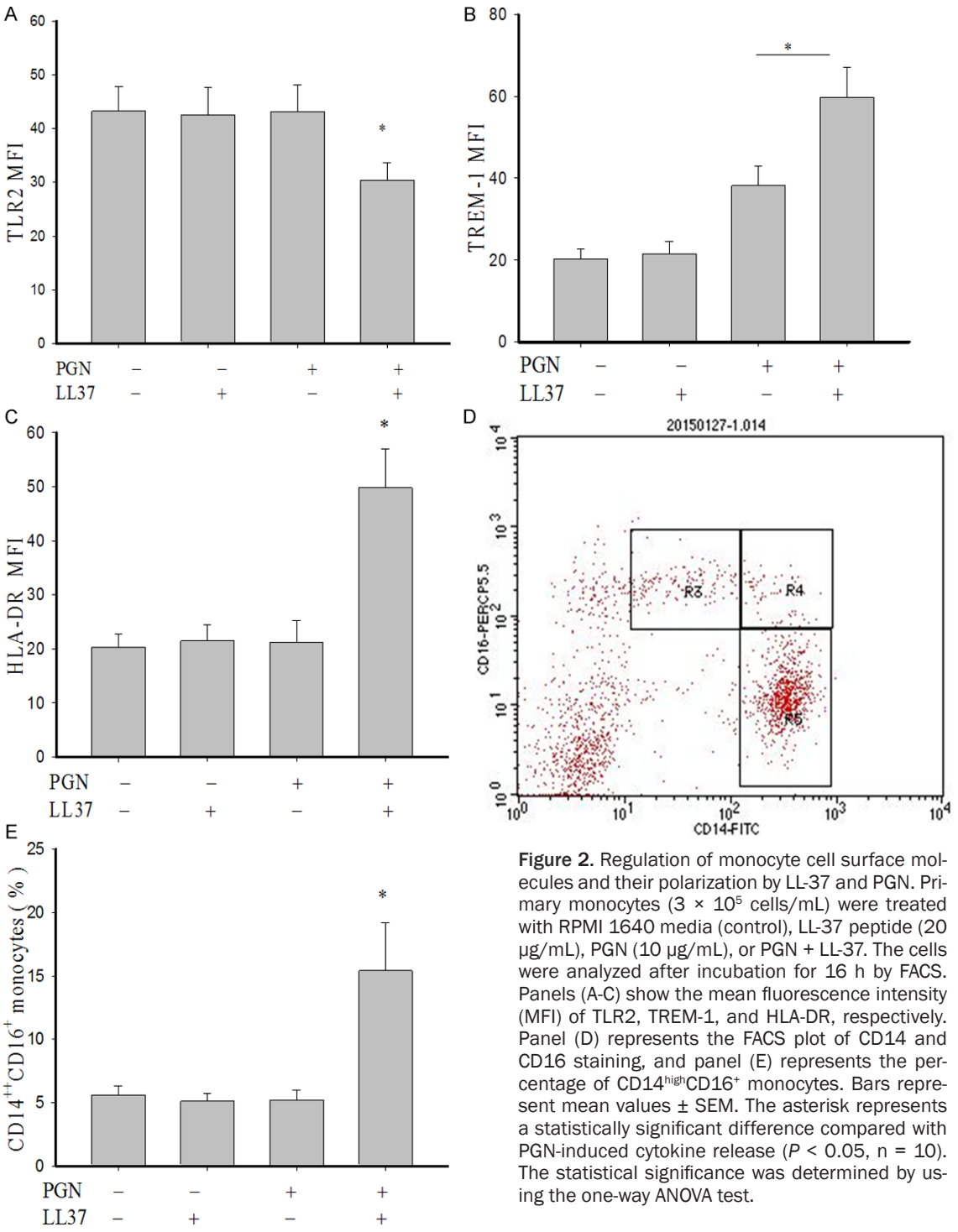

Figure 2. Regulation of monocyte cell surface molecules and their polarization by LL-37 and PGN. Primary monocytes (3×10^5 cells/mL) were treated with RPMI 1640 media (control), LL-37 peptide (20 μg/mL), PGN (10 μg/mL), or PGN + LL-37. The cells were analyzed after incubation for 16 h by FACS. Panels (A-C) show the mean fluorescence intensity (MFI) of TLR2, TREM-1, and HLA-DR, respectively. Panel (D) represents the FACS plot of CD14 and CD16 staining, and panel (E) represents the percentage of $CD14^{high}CD16^{+}$ monocytes. Bars represent mean values ± SEM. The asterisk represents a statistically significant difference compared with PGN-induced cytokine release ($P < 0.05$, n = 10). The statistical significance was determined by using the one-way ANOVA test.

a typical phenotype of monocyte-derived iDCs. In addition, the cells treated with both LL-37 and PGN displayed markedly higher expression of CD86 and major histocompatibility complex (MHC) class II molecules (such as HLA-DR), which are involved in antigen presentation and provide a costimulatory signal for T cell activation. Simultaneously, monocyte cells were also treated with GM-CSF and IL-4 (as shown in **Figure 3A**, last column) to be used as a positive control for comparison as they induce the generation of iDCs.

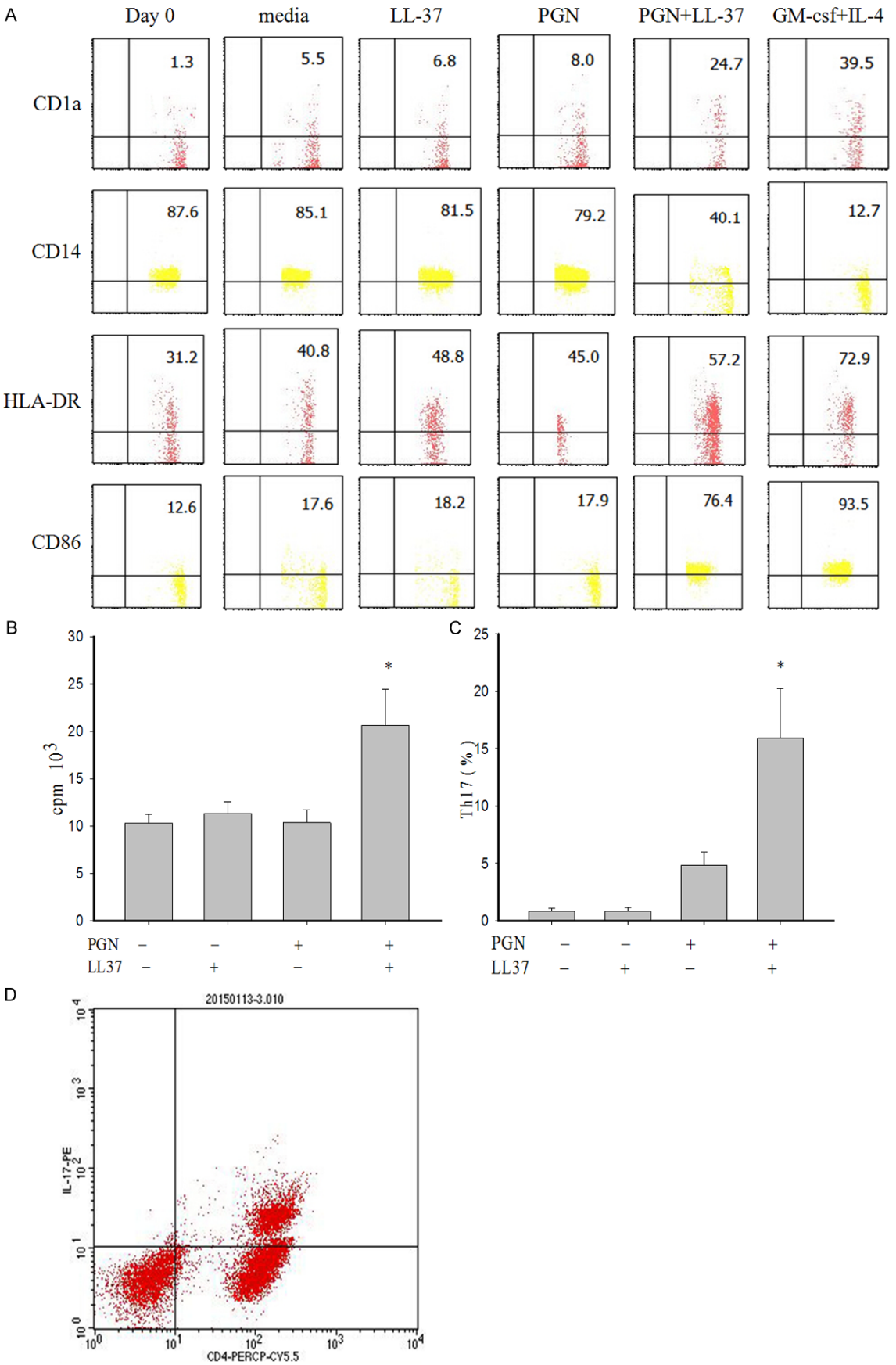
A
Day 0
media
LL-37
PGN
PGN+LL-37
GM-csf+IL-4
CD1a
1.3
5.5
6.8
8.0
24.7
39.5
CD14
87.6
85.1
81.5
79.2
40.1
12.7
HLA-DR
31.2
40.8
48.8
45.0
57.2
72.9
CD86
12.6
17.6
18.2
17.9
76.4
93.5
B
cpm 10^3
PGN
LL37
C
Th17 (%)
PGN
LL37
D
20150113-3.010
IL-17-PE
CD4-PERCP-CY5.5

Figure 3. LL-37 and PGN regulate primary monocyte differentiation into iDCs and T cell proliferation. Primary monocytes (3×10^5 cells/mL) were treated with RPMI 1640 media (control), LL-37, PGN, or LL-37 + PGN for 5 days. A small portion of these monocytes were also cultured with GM-CSF and IL-4 to derive iDCs for comparison (positive control). A. Represent the staining of CD1a, CD14, HLA-DR, and CD86 as analyzed by flow cytometry at 5 days after treatment. The first column is a control from day 0, without any treatment; and the last column (GM-CSF + IL-4 treatment) represents a positive control for iDCs. Each plot represents the percentage of cells that stained positive for the marker indicated relative to the intensity of the isotype control staining (horizontal line). The results shown are representative from one experiment among three independent experiments. B. Irradiated iDCs (1×10^4 cells/well) were cocultured with purified T cells from an unrelated donor for 3 days in a MLR assay. After 3 days, T cell proliferation was measured by [^{3}H] thymidine incorporation as described in the Materials and Methods section. C. Bars represent the percentage of Th17 staining (measured by IL-17 cytokine staining) after monocyte treatment as described in the Materials and Methods section. D. The FACS plot representing the IL-17 staining. Error bars represent SEM from triplicate samples. The asterisk represents a statistically significant difference compared with PGN-induced cytokine release ($P < 0.05$, n = 3). The statistical significance was determined by using the one-way ANOVA test.

LL-37 and PGN-induced iDCs efficiently stimulate T cell proliferation and Th17 polarization

To further understand the functionality of LL-37 and PGN-induced iDCs, we tested their ability to function as APCs in an MLR assay. Monocytes were cultured with PGN and LL-37 as well as the relevant controls for 5 days to induce the iDC phenotype. After the addition of purified T cells from an unrelated donor at a ratio of 1:20 (iDCs/T cells) and coculturing for 3 days, T cell proliferation was measured. The results showed that monocytes induced by the combination of LL-37 and PGN promoted a dramatic increase in T cell proliferation (**Figure 3B**) and Th17 polarization (**Figure 3C**, **3D**), compared to cells activated with PGN or LL-37 alone.

Analysis of monocyte subpopulations from psoriasis guttata patients

The psoriasis patient samples exhibited a greater proportion of $CD14^{high}CD16^{+}$ monocytes, compared to healthy controls (**Figure 4A-D**). However, this intermediate $CD14^{high}CD16^{+}$ monocyte phenotype of patients displayed a lower expression of TREM-1 than the healthy controls (**Figure 4E**, **4F**). Interestingly, in psoriasis guttata patients, sTREM-1, which is liberated by cleavage of the extracellular domain of TREM-1 [32], appeared to be elevated when compared to the healthy control group (**Figure 5A**). Moreover, the serum LL-37 levels were also elevated in patients with psoriasis compared to healthy controls (**Figure 5B**). In addition, the percentage of $CD14^{high}CD16^{+}$ monocytes showed a positive correlation with the serum levels of sTREM-1 ($r = 0.525$; $P < 0.05$) and anti-streptolysin O (ASO) ($r = 0.521$; $P < 0.05$) as well as the PASI scores ($r = 0.504$; $P < 0.05$) in patients with psoriasis guttata (**Figure 5C-E**). However, no correlation was detected for LL-37 (**Figure 5F**), C-reactive protein, the erythrocyte sedimentation rate, or the white blood cell count (data not shown).

Discussion

Excessive production of LL-37 represents a typical abnormality in the skin lesions of psoriasis patients. In recent years, LL-37 has been highlighted as a modulator of psoriasis development. The human LL-37 peptide participates at the interface of innate and adaptive immunity by regulating cytokine and chemokine production by a range of cell types and chemoattraction of various immune effector cells [33]. Simultaneously, inflamed skin in psoriasis also has been shown to have increased numbers of PGN-containing macrophages [12]. In this study, we demonstrated that LL-37 modulated PGN-induced inflammatory monocytes and induced their polarization towards the $CD14^{high}CD16^{+}$ subset, eventually causing them to differentiate into iDCs. These iDCs not only displayed enhanced expression of CD1a, CD86, and MHC class II molecules but also showed an improved ability to elicit T cell proliferation and Th17 production.

The idea that TREM-1 induction by PGN in combination with LL-37 might play a central role in monocyte polarization and differentiation is supported by recent studies. For example, it has been shown that TREM-1 modulates macrophage polarization toward the pro-inflammatory M1 type [34]. This finding was further validated by the data, suggesting that GM-CSF-derived M1 macrophages have high levels of TREM-1. Previously, other studies also have

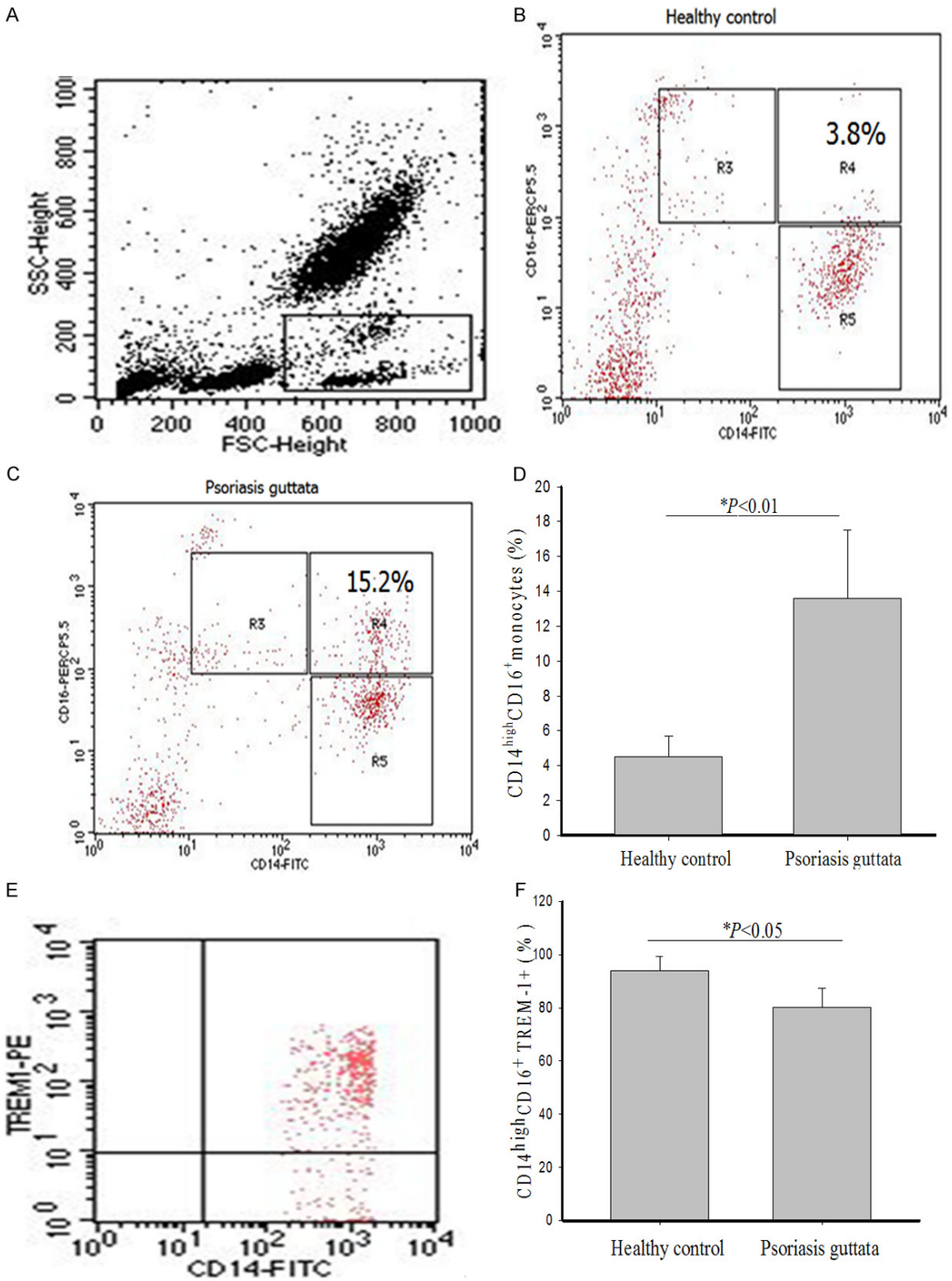

Figure 4. Analysis of monocyte subpopulations from psoriasis guttata patients. A. Representative flow cytometry plot showing forward and side scatter characteristics with gating used to identify monocytes (R1). B and C. Plots showing CD14 and CD16 expression on gated monocytes from healthy controls and psoriasis guttata patients, respectively. D. Percentage of $CD14^{high}CD16^{+}$ monocytes from healthy controls and patients with psoriasis. E. Plot showing TREM-1 expression on $CD14^{high}CD16^{+}$ monocytes (R4). F. Percentage of TREM-1-positive $CD14^{high}CD16^{+}$ monocytes from healthy controls and psoriasis guttata patients. The T-test was used to analyze differences between the two groups. The asterisk represents a statistically significant difference compared with the control group ($P < 0.05$).

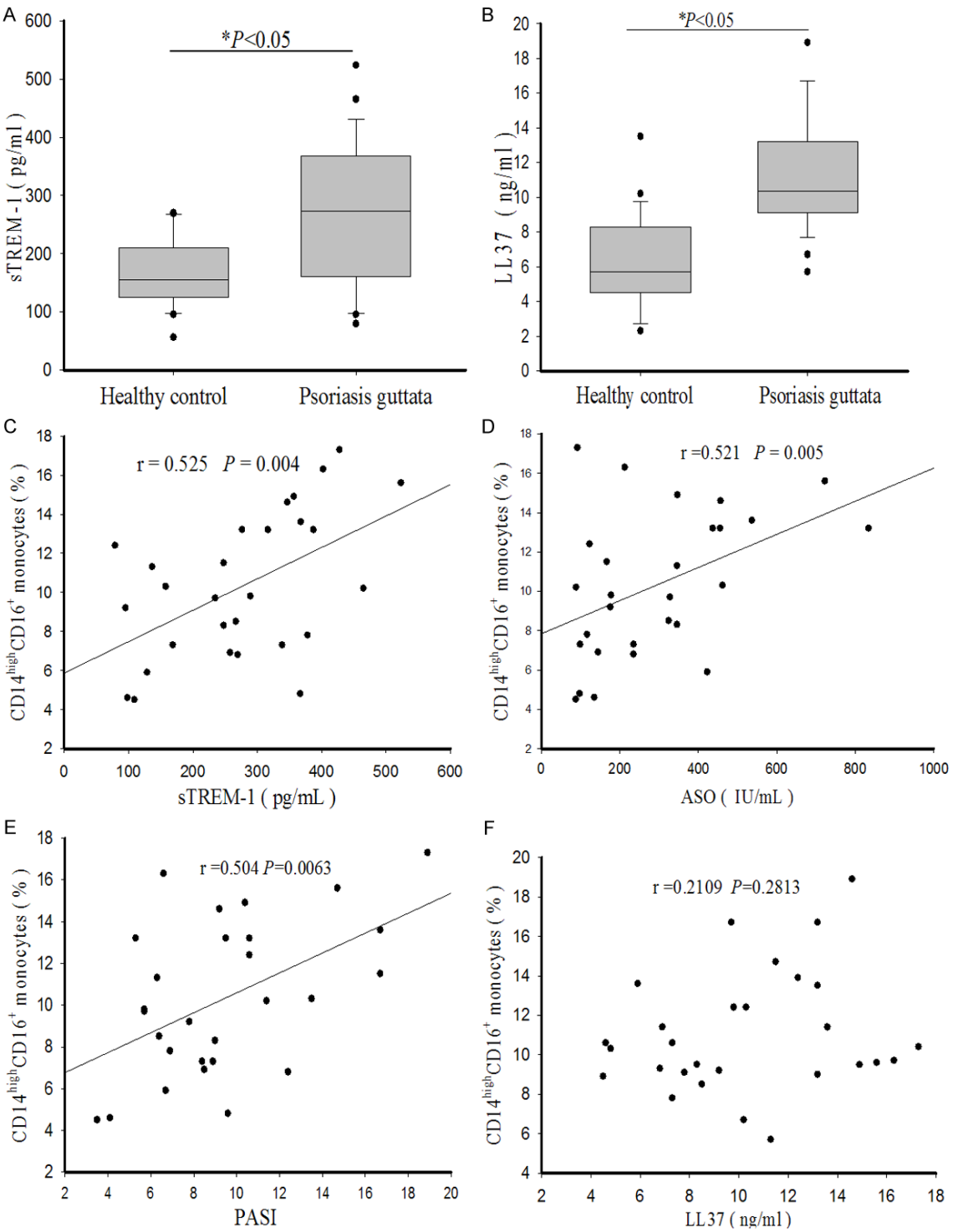

Figure 5. Comparison of certain markers in serum samples of psoriasis guttata patients and healthy controls and their relationship with $CD14^{high}CD16^{+}$ monocytes. A and B. Represent the comparison between the levels of sTREM-1 and LL-37 in healthy controls and psoriasis guttata patients. Statistical significance was determined by the Mann-Whitney U test. The asterisk represents a statistically significant difference compared with the control group ($P < 0.05$). C-F. Represent correlations between $CD14^{high}CD16^{+}$ monocytes and the serum levels of sTREM-1 and ASO, the PASI scores, and the levels of LL-37 in patients with psoriasis guttata. "r" denotes the Pearson correlation coefficient, and the *P* value represents statistical significance.

shown that TREM-1 can induce robust production of GM-CSF [32]. In our study, we also observed the significant upregulation of GM-CSF. In addition, accumulating evidence indicates the involvement of TREM-1 activation in directing monocyte differentiation into iDCs. These data are supported by the induction of higher expression of CD1a, CD86, CD83, and MHC class II molecules [35]. Furthermore, TREM-1 also has been suggested to play a central role in inducing the generation of monocyte-derived myeloid dendritic cells in a hypoxic environment [36].

In addition, LL-37 has been shown to be involved in monocyte/macrophage differentiation [37]. Davidson *et al*. have reported that LL-37 enhances the GM-CSF/IL-4-driven differentiation of blood monocytes into immature DCs [38]. The LL-37-induced DCs displayed a significantly higher endocytic capacity, modified phagocytic receptor expression/function, and upregulated surface levels of CD86, CD11b, CD11c, and CD18. Similarly, other studies have consistently shown that other antimicrobial peptides/proteins, such as hLF1-11, can drive monocyte differentiation toward DCs that enhance Th17 polarization [39]. Human defensin and human neutrophil peptide-1 also have promoted the activation and maturation of DCs as well as stimulated the production of TNF-α, IL-6, and IL-12 [40].

Similarly, in this study, we observed that PGN in combination with LL-37 drove monocyte differentiation toward iDCs, resulting in Th17 polarization, an influx of neutrophils, and the production of cytokines/chemokines and antimicrobial peptides by epithelial cells. The results also suggested that skin lesions in psoriasis patients have abundant myeloid dendritic cells, and they strongly express the MHC class II molecule, which is responsible for intense stimulation of T lymphocytes due to the pronounced release of IL-12 [41].

Furthermore, analyses of monocytes in psoriasis guttata patients revealed that the majority of them are the intermediate type with the $CD14^{high}CD16^{+}$ phenotype; this phenotype was significantly more prevalent in the psoriasis guttata patients than corresponding healthy controls. Also, the psoriasis guttata patients had higher circulating levels of sTREM-1. These results are consistent with the earlier findings of $CD14^{high}CD16^{+}$ monocytes, sTREM-1, and LL-37 in psoriasis guttata patients [18, 42, 43]. Recent studies also have suggested that intermediate monocytes with high APC function express high levels of the MHC class II molecules HLA-ABC and HLA-DR as well as the costimulatory molecule CD40 [44]. Moreover, adsorptive depletion of $CD14^{high}CD16^{+}$ proinflammatory monocyte phenotype has been shown to improve the clinical outcome of patients with pustular psoriasis, suggesting their important role in psoriasis [43].

In addition, our results further indicated the positive correlations between sTREM-1 and the frequency of $CD14^{high}CD16^{+}$ cells, but we did not observe an increased TREM-1 expression on monocytes. This finding can be explained by the fact that when TREM-1 is upregulated, it is released into bodily fluids as sTREM-1. Many studies have indicated that sTREM-1 could be a valuable diagnostic biomarker for various infectious diseases [45, 46]. Also, the serum LL-37 levels were elevated in patients with psoriasis compared with healthy controls, but its levels did not correlate with the percentage of $CD14^{high}CD16^{+}$ monocytes. One possible explanation is that the serum LL-37 level represents the sum total of LL-37 produced by various cells from the intestine, airways, lymph nodes, and bone marrow. In fact, the serum LL-37 level has been suggested to be affected by immune activities of multiple organs as well as many other factors such as vitamin D levels or ultraviolet and microbial exposure in the environment [43].

In conclusion, this study has demonstrated that LL-37 in synergy with PGN can induce monocyte polarization and differentiation into a proinflammatory phenotype *in vitro*. It would be interesting to further evaluate the mechanism behind this induction due to the potential pathogenic importance of the $CD14^{high}CD16^{+}$ monocyte activation pathway in driving inflammatory responses in psoriasis guttata. Moreover, some additional studies are required to determine whether the $CD14^{high}CD16^{+}$ subset could potentially be used as a prognostic or predictive biomarker to treat psoriasis guttata and whether modulation of monocytic polarization represents an attractive target for future therapeutic interventions.

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Disclosure of conflict of interest

None.

Abbreviations

TREM-1, triggering receptor expressed on myeloid cells-1; MFI, mean fluorescence intensity; PGN, Peptidoglycan; iDCs, immature dendritic cells; IL, interleukin; TNF-α, tumor necrosis factor-α; sTREM-1, soluble form of triggering receptor expressed on myeloid cells-1; PASI, Psoriasis Area and Severity Index; PBS, Phosphate-buffered saline; GM-CSF, granulocyte-macrophage colony-stimulating factor; MLR, Mixed lymphocyte reaction; ASO, antistreptolysin O.

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