Cellular Immunology 337 (2019) 62–70

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

Biological activities of interleukin (IL)-21 in human monocytes and macrophages

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ARTICLE INFO

Keywords:
Interleukin-21
Monocytes
Macrophages
Cellular functions

ABSTRACT

The biological roles of interleukin (IL)-21 in human monocytes and macrophages have been neglected. We previously demonstrated that IL-21 induce phagocytosis and established that Syk is a new molecular target of IL-21. Herein, we found that IL-21 is not chemoattractant for immature THP-1 and primary monocytes but can increase the capacity of THP-1 cells (not primary monocytes) to adhere onto a cell substratum by a Syk-dependent mechanism without altering the expression of a panel of cell surface molecules. Unlike THP- 1 and monocytes, IL-21 can increase metalloproteinase (MMP)-9 secretion and activity in monocyte-derived macrophages (HMDM), as assessed by western blot and zymography experiments, respectively. We reported that IL-21 did not increase the production of IL-6 and the chemokines MIP-1α and GRO-α in HMDM. Therefore, IL-21 can increase functions other that phagocytosis, but this cytokine does not have a large spectrum of biological activities in monocytes and macrophages.

1. Introduction

Interleukin (IL)-21 is the most recent identified member of the CD132 or γc user cytokines [1–3]. It is mainly produced by activated T and NKT cells and plays a crucial role in the regulation of immune functions [4]. Its receptor is composed of a specific alpha chain (IL-21Rα or CD360) and the common γ chain (γc or CD132) shared by receptors to IL-2, IL-4, IL-7, IL-9 and IL-15. The binding of IL-21 to its receptor, leads to the activation of several signaling pathways, including, Janus kinase (JAK) and signal transducer and activator of transcription [5] pathways [1,3,5–7], mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt pathway [8,9]. IL-21 has a pleiotropic effect on several cell types as it can mediate T cell survival, their differentiation and proliferation, promote the maturation of B cells into plasma cells and immunoglobulin (Ig) producing cells, and regulate NK cell proliferation and cytotoxicity [4]. More recently, few studies demonstrate that IL-21 could also affect the biology of cells of myeloid origin. For example, IL-21 possesses inhibiting activities on bone marrow derived dendritic cells since it can reduce their activation, maturation and reverse the cytokine production induced by LPS stimulation [10,11]. In contrast, one other study reported that IL-21 induced IL-1β in conventional DC that was partially regulated by STAT3 activation [12]. Thus, IL-21-induced responses can vary between similar cell types, underlining the needs for better understanding its effect among all different types of cells. In this respect, the role of IL-21 on monocytes and macrophages has been less studied. We are among the few teams investigating in this area and we have demonstrated that IL-21 enhances the ability of primary human monocytes and granulocyte macrophage-colony stimulating factor (GM-CSF)-derived macrophages to exert phagocytosis and that spleen tyrosine kinase [13] is a new intracellular molecular target of IL-21 [14,15].

IL-21 is involved in the development of several inflammatory and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel diseases [16–19]. Despite the fact that the role of IL-21 in inflammatory diseases was evidenced by the utilization of a blocking IL-21R. Fc fusion protein where the severity and symptoms were diminished, the exact mode of action is still not clear. In one study, IL-21 was found to induce migration and invasion of fibroblast-like synoviocytes (FLS) from rheumatoid arthritis (RA) patients as well as metalloproteinase (MMP)-3 and MMP-9 secretion via PI3K/Akt, STAT and MAPK pathways, leading to bone and cartilage deterioration [20]. In one other study, IL-21 was found to induce MMP-1, -2, -3 and -9 expression and activity in fibroblasts from Crohn disease patients [21] and MMP-2 and -9 *Helicobacter pylori*-infected gastric mucosa [22]. Also, both invasion and migration of cancerous MDA-231 cells were promoted by IL-21 [23]. Monocytes and macrophages are known to express a functional IL-21R and knowing the importance of MMPs in disease progression, we investigate here if IL-21 could alter

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https://doi.org/10.1016/j.cellimm.2019.02.002
Received 17 September 2018; Received in revised form 31 January 2019; Accepted 8 February 2019
Available online 08 February 2019
0008-8749/

function others than phagocytosis, including MMP secretion/activity in these cells.

2. Materials and methods

2.1. Reagents

RPMI 1640, HEPES, penicillin/streptomycin, heat inactivated fetal bovine serum (FBS) and Hank's balance salt solution (HBSS) were purchased from Life technologies (Grand Island, NY, USA). Ficoll-paque was obtained from GE healthcare Bio-science AB (Uppsala, Sweden). Trypan blue, the JAK-2/JAK-3, STAT-1, -3, -5a and -5b inhibitor tyrphostin B42 (or AG490), MAPK/ERK inhibitor PD98059 and p38 MAPK inhibitor SB203580 were obtained from Sigma Aldrich (St-Louis, MO, USA) and the Akt inhibitor wortmannin and Syk inhibitor II were purchased from EMD Biosciences (San Diego, CA, USA). Calcein-AM was from Invitrogen (Eugene, OR, USA). Cytokines (GM-CSF and IL-21) were purchased from Peprotech (Rocky Hill, NJ, USA). Antibodies against CD11a, CD11b, CD29, CD62L, ICAM-3 and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-CD18, -CD49d and – ICAM-1 were from BD Biosciences (San Diego, CA, USA). Antibodies against MMP-2 and MMP-9 were bought from (Abcam, Cambridge, UK). Ultrapure LPS from Salmonella spp. was purchased from InvivoGen (San Diego, CA). All secondary antibodies came from Jackson Immuno Research Laboratories (West Grove, PA).

2.2. Cell culture conditions and preparation of human macrophages

2.2.1. THP-1 cell culture

THP-1 immature monocytes (ATCC® TIB-202™) were obtained from ATCC (American Type Culture Collection, Manassas, VA), and culture in RPMI 1640 supplemented with 2.05 mM L-Glutamine, 100 mM HEPES, 100 U/ml penicillin, and 100 mg/ml streptomycin (further referred to as RPMI 1640) and 10% FBS and incubated in a 5% CO_2 atmosphere at 37 °C. Cells were subculture before reaching a concentration of 8×10^5 cells/ml as recommended by ATCC. Cell viability was systematically evaluated by trypan blue exclusion assay before and after each treatment, and mortality never exceeded 5%.

2.2.2. EA.hy926 cell culture

The human umbilical vein cell line EA.hy926 (ATCC® CRL-2922™) was purchased from ATCC and was grown in RPMI-1640 supplemented with 10% FBS as we previously documented [24]. Cell viability was systematically evaluated before and after each treatment, and mortality never exceeded 5%.

2.2.3. Human macrophages

Human peripheral blood mononuclear cells (PBMC) were isolated from venous blood of healthy volunteers by centrifugation over Ficoll-paque. Blood donations were obtained from informed and consenting individuals by an institutionally approved procedure. Human monocyte-derived macrophages (HMDM) were generated by incubating 4×10^6 PBMC at 37°C in a 5% CO_2 atmosphere for 2 h in RPMI-1640 medium supplemented with 10% heat-inactivated human autologous serum in 48-well plates. Monocytes obtained by removing the non-adherent PMBC were further incubated for 7 days in RPMI- 1640 medium supplemented with 10% heat-inactivated FBS + 2 ng/ml GM-CSF to obtain macrophages as previously described [14]. In all experiments, cell viability was assessed by trypan blue exclusion prior to experiments and mortality never exceeded 5%.

2.3. Adherence assay

Human monocyte-like THP-1 cells were treated with the diluent or with IL-21 (50 ng/ml) for 30 min, labeled for 30 min with 5 mM calcein-AM and coculture onto confluent EA.hy926 endothelial for 1 h. Wells were rinsed five times with HBSS an adhesion of THP-1 cells was determined by observation with a Leica DMIL photomicroscope equipped with an ebq 100 dc epifluorescent condenser. Images were taken with a Canon Eos. Results were obtained by counting five representative fields using Image J cell counter. In some experiments, THP-1 cells were pretreated with inhibitors to MAPK/ERK (PD98059, 10 μM), MAPK p38 (SB203580, 5 μM), Jak/STAT (AG490, 30 μM), PI3K/Akt (wortmannin, 50 nM), or Syk (Syk inhibitor II, 1 mM) for 30 min prior to the adherence assay.

2.4. Assessment of adhesion molecule expression by flow cytometry

THP-1 cells (1×10^6 cells/ml) was incubated in PBS-20% human serum to block Fc site. After several washes, cells were stained with 10 μl of FITC-conjugated anti-CD11a, -CD11b, -CD62L, ICAM3 or PE-conjugated anti-CD18, -CD29, -CD49d or the appropriate isotypic control antibodies for 30 min on ice. Cells were washed twice with ice-cold PBS before being resuspended at 1×10^6 cells/ml. Cell surface expression was determined immediately using a fluorescence activated cell sorter (FACS)Calibur (BD Biosciences, San Jose, CA, USA). In some experiments, cells were treated for 30 min with the diluent or IL-21 (50 ng/ml) and cells were marked as above.

2.5. Human monocyte-like THP-1 chemotaxis assay

In vitro chemotaxis was performed in a 48-well microchemotaxis chamber (Neuro Probe Inc., Gaithersburg, MD) using a 5 μm polycarbonate membrane filter as previously described [25]. Briefly, the bottom wells were loaded with diluent, increasing doses of IL-21 (5–500 ng/ml) or LPS (100 ng/ml). The membrane was then placed over the wells, and the top layer of the chamber was finally added over the membrane. 5×10^4 Cells (50 μl from a 1×10^6 cells/ml in RPMI-1640 suspension) were added to the top chamber wells and was incubated at 37 °C for 60 min in a humidified incubator in 5% CO_2 atmosphere. After the incubation, the top of the chamber was removed and the upper side of the membrane was carefully wiped with the rubber scraper provided by the manufacturer. Finally, the membrane was stained with Hema-3 Stain staining kit according to the manufacturer's instruction and mounted on a glass slide, and examined under a microscope 400X. The number of cells in 10 representative fields was counted and the results were expressed as the absolute number of cells.

2.6. Zymography

THP-1 cells or HMDM were treated as indicated in the figure legend for 24 h and the samples were then centrifuged at 2000 rpm for 10 min at 4C. The supernatants (10 μl) were then mixed with non-reducing buffer (40% glycerol, 1 M Tris-HCl [pH 6.8], 8% sodium dodecyl sulfate [SDS]) and resolved over a 7.5% acrylamide gel containing 0.2% gelatin. The gel was then washed for 30 min twice with 2.5% Triton X-100 in water and incubated overnight in enzymatic digestion buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 5 mM $CaCl_2$). Thereafter, the gels were stained with 0.1% Coomassie blue solution and de-stained to determine the gelatinase activity, i.e. visualized by the apparition of white zones in blue-stained gel.

2.7. Western blotting experiments

Pellets from cells treated for zymography were lysed in Laemmli's sample buffer and resolved onto 7.5% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes for the detection of specific protein as indicated in the figure. Membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS)-Tween (0.15%) containing 3% BSA. After washing, primary antibodies were added at a final dilution of 1:1000 in TBS-Tween. The membranes were kept overnight at 4 °C with gentle

agitation, then washed with TBS-Tween and incubated for 1 h at room temperature with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1:20,000 in TBS-Tween) followed by several washes. Protein expression was revealed using Clarity ECL substrate with ChemiDoc MP imaging systems (Bio-Rad, Hercules, CA, USA). Membranes were stripped with reblot plus strong antibody stripping solution (Millipore, Billerica, MA, USA) and reprobed to confirm equal loading of proteins.

2.8. Production of cytokines/chemokines by human macrophages

HMDM were rinsed twice with warm HBSS and then incubated with diluent, LPS (100 ng/ml), IL-21 (50 ng/ml) or LPS + IL-21 in RPMI 1640 medium-10% heat-inactivated FBS, for 24 h in a 5% CO_2 atmosphere at 37 °C. The supernatants were then collected and centrifuged at 13,000 g for 10 min at 4 °C and then stored at −80 °C for. The concentration of IL-6, MIP-1α and GRO-α released in the external milieu were quantified using commercial ELISA kits according to the manufacturer's instructions (IL-6 and MIP-1α from Life Technologies, Frederick (MD) and Gro-α from R&D Systems, Minneapolis (MN)).

2.9. Statistical analysis

The data are reported as mean ± SEM and were analyzed by one-way ANOVA (repeated measures ANOVA) and differences of each group vs. control groups were assessed by the Dunnett or Tukey's post-test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). Statistical significance was established at $^*p < 0.05$.

3. Results

3.1. IL-21 does not induce migration of immature THP-1 and primary monocyte cells

As monocyte recruitment to an inflame site is a major step for establishing inflammation and, since IL-21 possesses the capacity to induce migration [20,23], we asked whether or not IL-21 would induce migration in THP-1 cells. As illustrated in Fig. 1A and B, IL-21 does not induce chemotaxis at various tested concentrations ranging from 5 to 500 ng/ml. In contrast, and, as expected, LPS (100 ng/ml) induced chemotaxis by a factor of ~3. Similarly, IL-21 did not induce chemotaxis in primary monocytes (Fig. 1C). Because of the variation among donors, the results were rather expressed as ratios (indices, where Ctrl was set as 1).

3.2. IL-21 induces adhesion of THP-1 cells onto endothelial EA.hy926 cells by a Syk-dependent mechanism

Next, we verified if IL-21 could increase cellular adhesion. Fig. 2 shows that IL-21 increases the capacity of THP-1 cells to adhere onto endothelial EA.hy926 cells by a factor of 2.3 ± 0.4. In contrast, we did not observe this in primary monocytes where IL-21 did not alter the basal level of adhering cells (data not shown). Since we previously reported that Jak/STAT, MAPK, Akt and Syk activation was important in modulating other cellular functions by IL-21 in human monocytes and macrophages [14,15], and, knowing that these signaling pathways are also involved during monocyte adhesion [26,27], we next determined the importance of these pathways/enzymes in IL-21-induced cell adhesion. Using pharmacological inhibitors, we observed that the different inhibitors could slightly to moderately inhibit the IL-21 effect (Fig. 2B) although a tendency was noted for p38 (SB inhibitor), but that only Syk inhibition significantly reversed the IL-21 induced adhesion (Fig. 2C).

3.3. Surface expression of adhesion molecules in IL-21-induced THP-1 cells

Attachment of monocytes to the endothelial cell substratum requires interaction between adhesion molecules at the cell surface of both monocytes and endothelial cell [28–30]. Therefore, we next verified if treatment with IL-21 alters the THP-1 cell surface expression of different adhesion molecules. We first determined the basal expression of different adhesion molecules express at the surface of THP-1 cell. As shown in Fig. 3A, naïve THP-1 cells present a strong expression CD18, CD29, CD49D and CD11a and a weaker expression of ICAM1, ICAM3 and CD11b. As expected, THP-1 cells did not constitutively express

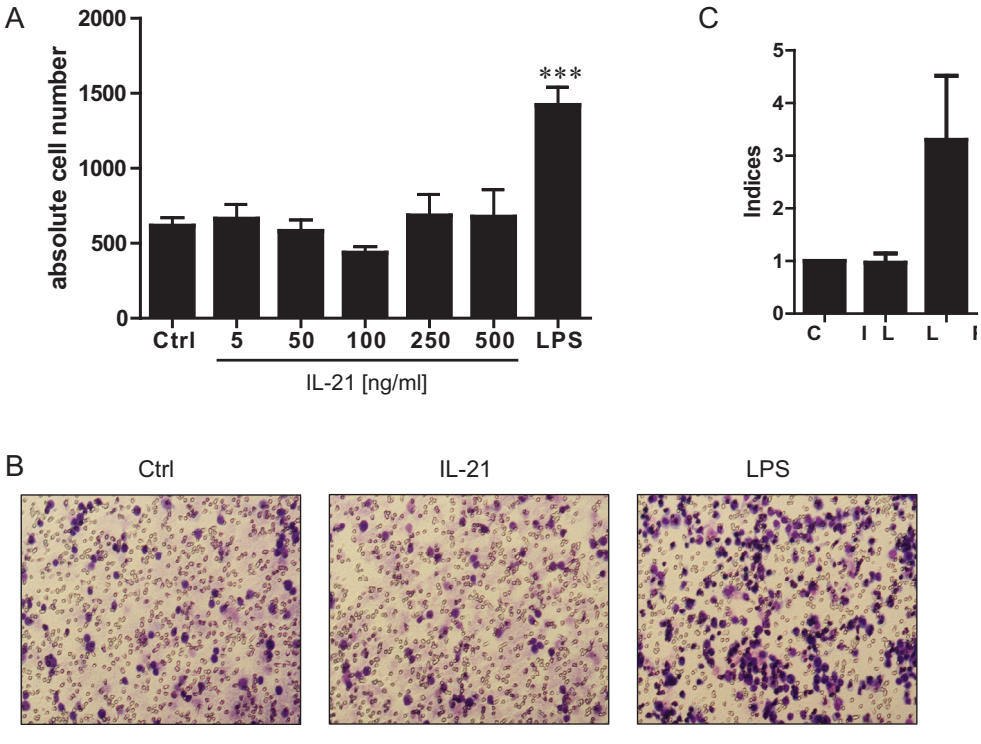

Fig. 1. IL-21 is not chemoattractant for immature human THP-1 monocytes and primary monocytes. THP-1 cells were incubated with diluent (Ctrl), the positive control LPS (100 ng/ml) or the indicated concentrations of IL-21 and freshly isolated primary monocytes were incubated with diluent, 50 ng/ml IL-21 or LPS in an *in vitro* chemotaxis chamber and chemotaxis was determined as described under Materials and Methods. A and C, results are expressed as absolute count number (A) or indices (C) and represented means ± SEM (n = 3). B, microphotograph of a representative field for Ctrl, IL-21 (50 ng/ml) and LPS.

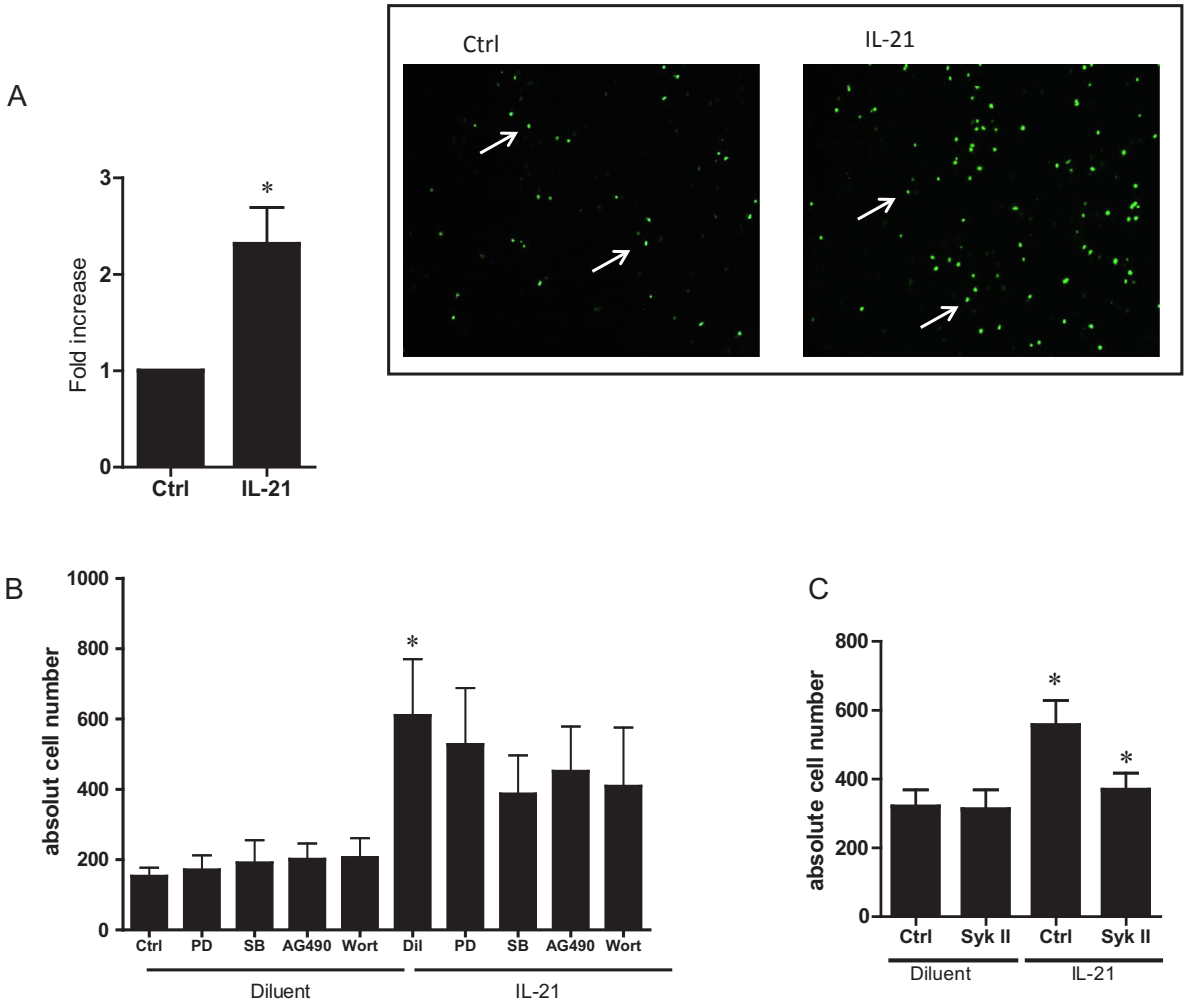

Fig. 2. IL-21 enhances adhesion of THP-1 cells onto endothelial EA.hy926 cells. THP-1 cells were treated with diluent (Ctrl) or IL-21 (50 ng/ml) for 30 min, labelled with calcein AM and incubated on confluent EA.hy926 cells for 1 h. Adhesion assay was performed and measured as described in Materials and Methods. A, results are expressed as fold of increase (means ± SEM, n = 6). Inset, Photomicrography of a representative field for Ctrl and IL-21 treated cells that were plotted in the bar graph. Dots, (arrows) represent adherent monocytes. B,C, cells were pretreated with MAPK/ERK inhibitor PD98059 (10 μM), MAPK p38 inhibitor SB203580 (5 μM), Jak/STAT inhibitor AG490 (30 μM), PI3K-Akt inhibitor wortmannin (50 nM), or Syk inhibitor II prior to the adhesion assay. Results are means ± SEM (B, n = 4 and C, n = 5).

CD62L at their cell surface [31]. Of note, the same treatment with IL-21 resulting in the increasing capacity of THP-1 to adhere onto endothelial cells did not alter the basal surface expression of any of the tested molecules (Fig. 3B–D). The same results were obtained even after 2 h of stimulation with IL-21 (*data not shown*).

3.4. Effect of IL-21 on gelatinase activity and MMP synthesis in monocytes and macrophages

3.4.1. IL-21 does not modulate LPS-induced MMP-9 secretion

Knowing that IL-21 can induce the secretion of MMPs in FLS, fibroblast and gut epithelial cells [20–22], and that it can also induce gelatinase activity, we first studied its ability to increase gelatinase activity by zymography experiments using the supernatants from IL to 21-induced THP-1 cells. Fig. 4A illustrates that, in contrast to LPS that induces MMP-9 (but not MMP-2) gelatinase activity, IL-21 (10–500 ng/ml) did not induce any of them. Using the same supernatants used for zymography experiments, LPS was found to markedly increase the MMP-9 (but not MMP-2, *data not shown*) protein expression as determined by western blot experiments. The corresponding cell pellets were used to determine the protein expression of both MMP-9 and MMP-2 and, as illustrated in Fig. 4B, LPS was found to increase the weak basal expression of MMP-9, unlike IL-21. In contrast to MMP-9, the intracellular basal expression of MMP-2 was relatively high, but IL-21 treatments did not alter its expression. As for THP-1 cells, IL-21 did not induce gelatinase activity by human primary monocytes (Fig. 4C). Of note, the MMP-2 was barely detectable (from absent to very weak) when using IL-21 treated primary monocyte supernatant. These results indicate that IL-21 did not induce gelatinase activity in both immature human THP-1 and primary monocyte cells.

Since IL-21 is known to reverse some LPS-induced activities, including the secretion of cytokines by human dendritic cells and murine macrophages [32,33], we next studied the capacity of IL-21 to modulate the LPS-induced gelatinase activity and MMP-9 expression in THP-1 cells. As shown in Fig. 5, IL-21 did not alter the LPS-induced gelatinase activity (Fig. 5A) as well as the protein expression of MMP-9 detected in the supernatants (Fig. 5B). Using the cell pellets to detect the intracellular MMP-2 and MMP-9 protein levels, we found that IL-21 did not change the levels induced by LPS treatment (Fig. 5C).

3.4.2. IL-21 induces gelatinase activity and MMP-9 secretion by human macrophages

Because of the above results indicating that IL-21 does not induce gelatinase activity, we next wanted to confirm this in HMDM. In

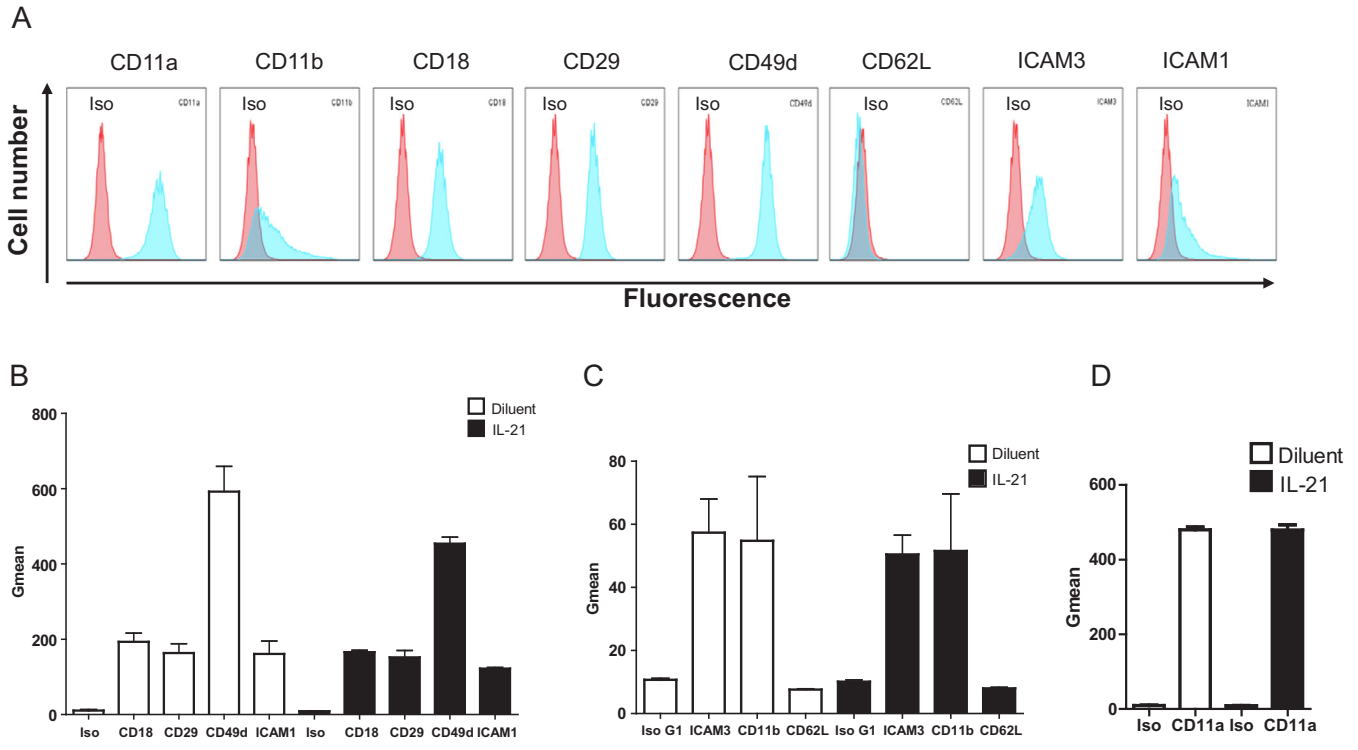

Fig. 3. IL-21 does not modulate cell surface expression of adhesion molecules in human monocyte-like THP-1 cells. Three separate batches of THP-1 cells were cultured and used to determine the cell surface expression of different adhesion molecules by flow cytometry using specific antibodies directed against CD11a, CD11b, CD18, CD29, CD49d, CD62L, ICAM-1 and ICAM-3 or the corresponding isotypic controls (Iso), as described in Materials and Methods. A, cells were untreated, treated with diluent or IL-21 (50 ng/ml) for 30 min. Note that the isotypic Ctrl overlapped with CD62L. B, results are expressed as geometric mean fluorescence intensity (Gmean). Data are means ± SEM (n = 3).

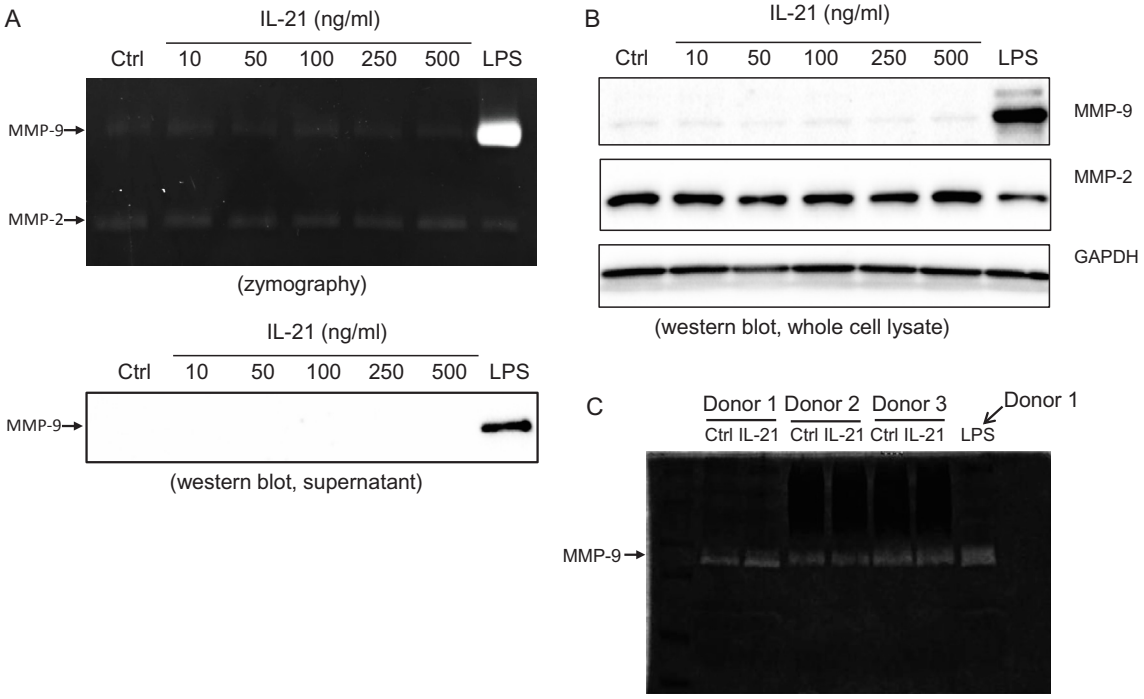

Fig. 4. IL-21 does not modulate the secretion of MMP-2 and MMP-9 and the gelatinase activity in human THP-1 and primary monocytes cells. THP-1 cells were treated with diluent (Ctrl), LPS (100 ng/ml) or the indicated concentrations of IL-21 for 24 h. A, gelatinase activity the supernatant was revealed by zymography as described in Materials and Methods (upper panel) and the presence of MMP-9 in the same supernatant was confirmed by western blot experiments (lower panel). Note that MMP-2 was undetectable in the supernatants (*not shown*). B, western bot analysis was used to detect the expression of MMP-2 and MMP-9 in the whole cell lysate. Results are from one representative experiment out of six. C, gelatinase activity was tested in three different experiments using the supernatants from untreated (Ctlr) or treated with IL-21 or LPS (donors 1, 2 and 3). Note that IL-21 does not increase gelatinase activity.

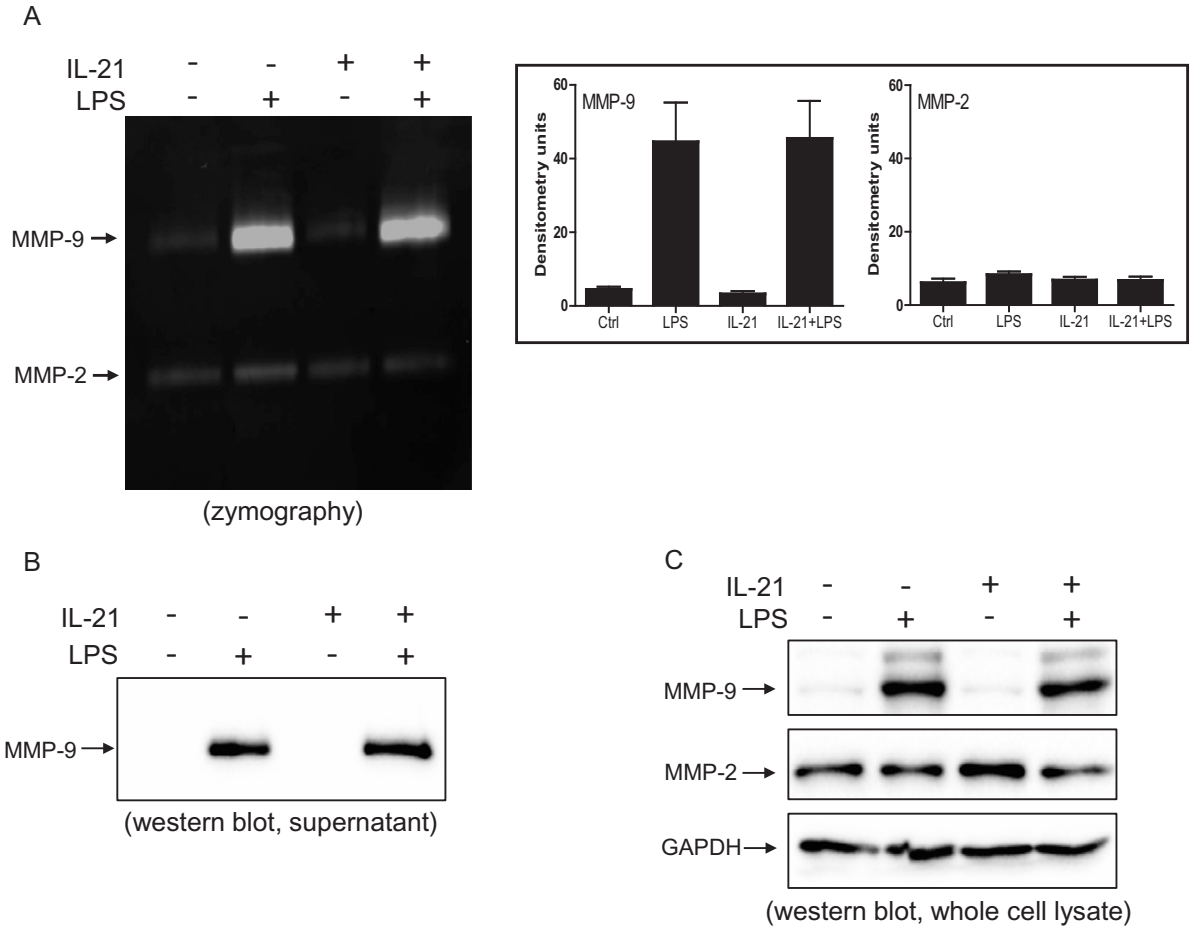

Fig. 5. IL-21 does not amplify the LPS-induced gelatinase activity and MMP-9 secretion in human THP-1 cells. THP-1 cells were treated with diluent, IL-21 (50 ng/ml), LPS (100 ng/ml) or IL-21 and LPS for 24 h. A, gelatinase activity was determined by zymography as described in Materials and Methods. Results are from one representative experiment out of six. Inset, the densitometric analysis of gelatinase activity of MMP-9 and MMP-2 are shown (means ± SEM, n = 6). The presence of MMP-9 and MMP-2 in the supernatant (B) or in the whole cell lysate (C) was determined by western blot experiments. Note that MMP-2 was undetectable in the supernatants (*not shown*). Results are from one representative experiment out of six.

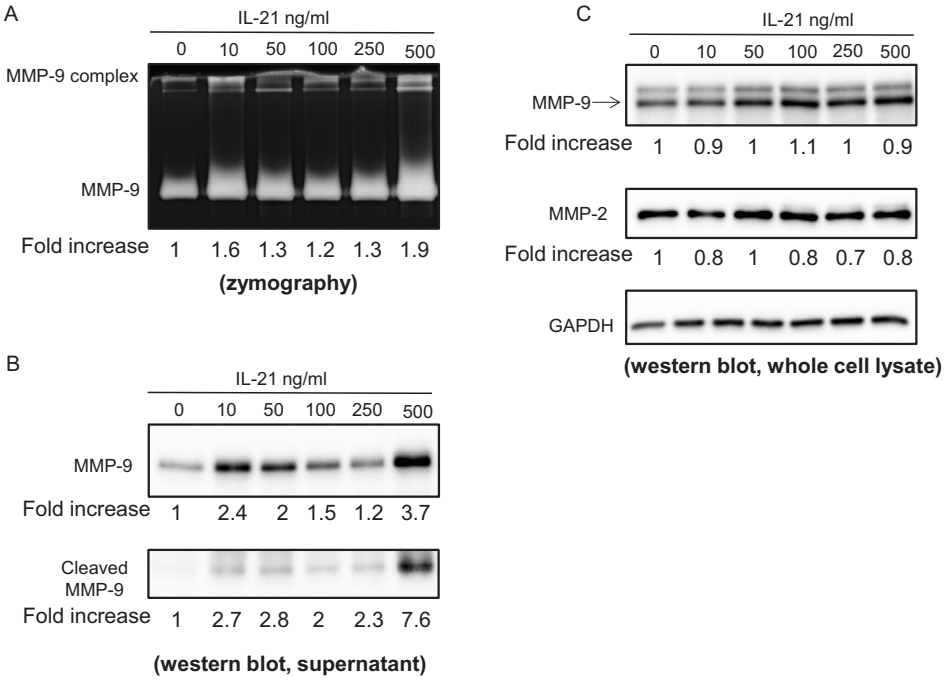

Fig. 6. IL-21 induces gelatinase activity and MMP-9 secretion in human macrophages. HMDM were treated with diluent (Ctrl) or the indicated concentrations of IL-21 for 24 h and gelatinase activity (A) or detection of MMP-9 and MMP-2 in the supernatants (B) or in whole cell lysates (C) were performed by zymography and western blot experiments, respectively. Note that MMP-2 was undetectable in the supernatants (*not shown*). Results are from one representative experiment out of three. Densitometric analyzes were performed using Quantity One, version 4.6.6 (Bio-Rad, Hercules, CA) and the results are expressed as fold increase vs Ctrl (set as 1) (panels A and B) and considering also GAPDH signals (panel C).

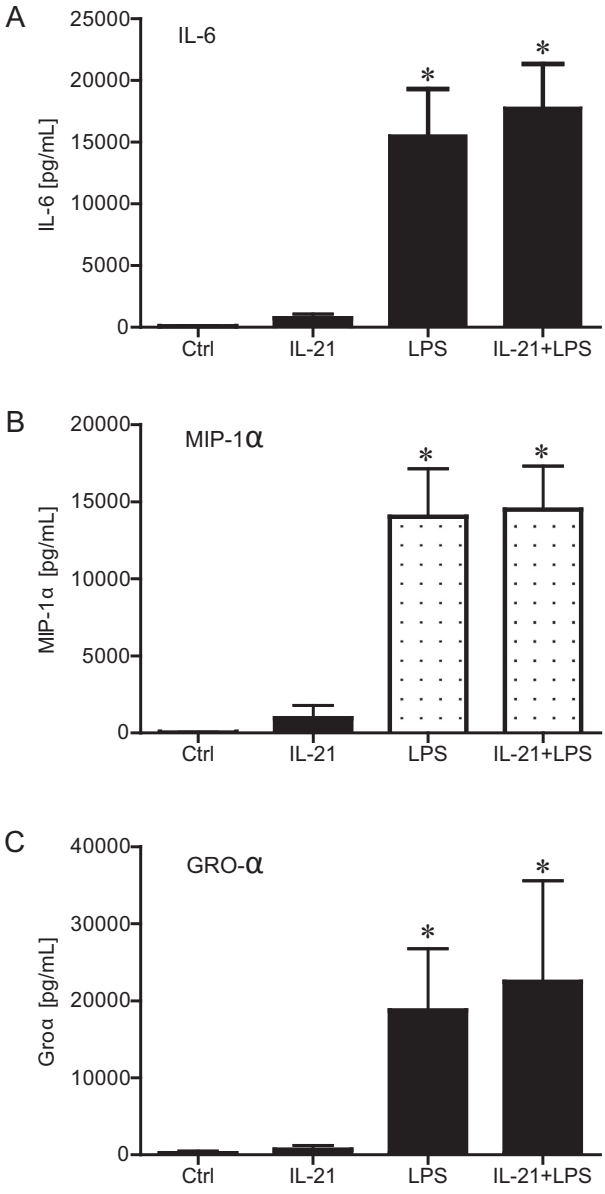

Fig. 7. IL-21 does not induce the production of IL-6, MIP-1α and GRO-α in naïve or in LPS-induced human macrophages. HMDM were treated with diluent (Ctrl), IL-21 (50 ng/ml), LPS (100 ng/ml) or a mixture of IL-21 + LPS for 24 h and the production of IL-6, MIP-1α and GRO-α was quantified by ELISA as described in Materials and Methods. Results are means ± SEM (n = 6).

contrast, as shown in Fig. 6A, IL-21 can induce gelatinase activity as determined by zymography experiments (upper panel) and can also induce the secretion of MMP-9 protein into supernatants (Fig. 6B) but not MMP-2 that, again, was undetectable in the supernatants as assessed by western blot (*data not shown*). As illustrated in Fig. 6C, IL-21 did not alter the intracellular the protein expression of both MMP-9 and MMP-2. Of note, as documented on the data sheet from the company, the antibody is known to (infrequently however) recognize two bands: one at 92–95 kDa (the pro-form) and one at 82 kDa (the active form of MMP-9, see arrow used for the densitometry).

3.5. IL-21 does not induce the production of IL-6, MIP-1α and GRO-α in naïve or in LPS-induced human macrophages

IL-21 is known to possess some pro-inflammatory activities [9,34]. Although it was previously found that IL-21 induced the production of different cytokines and chemokines in human monocytes [35], such role exerts in human macrophages is not well known. Fig. 7 illustrates that IL-21 did not alter the basal expression of the cytokine IL-6 (Fig. 7A) and of both chemokines MIP-1α and GRO-α (Fig. 7B and C, respectively). In addition, IL-21 did not alter the ability of LPS to increase the production of these soluble mediators in HMDM.

4. Discussion

IL-21 and other γc (CD132) user cytokines are known to be involved in a various inflammatory diseases, including RA, systemic lupus erythematosus and cancers [36,37]. The role of IL-21 in myeloid cell biology is becoming more defined. For example, it is well established that IL-21 can modulate monocytes and macrophages functions, especially phagocytosis and cytokine production [14,15,33,35,38], and to regulate dendritic cell maturation and functions [11]. Here, we show that IL-21, in contrast to LPS, is not a chemoattractant for immature human THP-1 and primary monocyte cells, but increases adhesion of THP-1 but not primary monocytes onto human endothelial cells. Adhesion of cells onto the endothelium is at the beginning of the process toward the migration of monocyte to the targeted site. Different cell surface adhesion molecules are involved during monocytes and endothelial cell interactions. For example, LFA-1, VLA-4 and Mac-1 are known to be involved in the monocyte rolling and arrest after their ligation to adhesion molecules, such as ICAM-1, ICAM-2 and VCAM-1 expressed at the surface of the endothelial cells [30]. Here, we show that IL-21 induces adhesion of the monocyte-like THP-1 cell line onto the EA.hy926 endothelial-like cell line without altering the cell surface expression of each of the individual component of LFA-1 (C18/CD11a), MAC-1 (CD18/CD11b) and VLA-4 (CD29/CD49d). In addition, we show that the cell surface expression of other adhesion molecules such as CD62L, ICAM-1 and ICAM-3 was also unaffected by IL-21 treatments. The ability of monocytes to adhere onto cell substratum depends on both the affinity and avidity of the adhesion molecules [39]. Therefore, our negative results might be explained by an increased affinity of some of these molecules or by a clustering of the integrins (valency) that could not be detected by our flow cytometry procedure. Also, we cannot rule out the possibility that other molecules expressed by monocyte are involved in IL-21-induced cell adhesion. For example, chemokine C—C motif receptor 1 (CCR1), CCR2 and CCR5 are known to be implicated in the arrest of monocytes [40], but have not been tested in our study. Finally, even though we did not directly stimulate the EA.hy926 cells, IL-21 could induce cytokine secretion by THP-1 cells which could activate the endothelial cells and in term increase expression of various adhesion molecules at their own surface and, therefore, help in the attachment of monocytes by an indirect mechanism. This remains to be determined. What is also unexplained is why IL-21 did not increase the adhesion of primary monocytes onto the same EA.hy926 cell substratum. However, these results clearly indicate that extrapolating results obtained with THP-1 cells to primary monocytes has to be taken with caution. For example, we previously reported that IL-21 can increase the production of IL-8 by HMDM, but not by primary monocytes and immature THP-1 cells, suggesting that mature differentiated cells are more responsive to IL-21 [15].

Several cell signaling pathways and kinases, including MAPK, PI3K/Akt, GPCR, and Syk are known to be involved in monocyte adhesion onto vascular epithelium [26–28]. Signaling events are important to increase the avidity by, for or example, mediating the conformational change of adhesion molecules needed to attach firmly to the vessel walls. We have previously shown that IL-21 can activate some cell signalling pathways in THP-1 cells [14,15]. In the present study, we used pharmacological inhibitors to evaluate the importance of Erk-1/2, Akt, p38 and Syk activation in IL-21-induced cellular adhesion. The partial inhibition observed after treatment with p38 and Syk inhibitors confirm that adhesion of monocytes onto endothelial cells is a complex process involving simultaneous activation of different cells signaling

pathways/kinases to be fully functional. However, our results also indicate that inhibition of only one signaling pathway was insufficient to completely reverse the IL-21 effect. Thus, these results suggest that even if IL-21 is not a direct chemoattractant for monocytes, it can help in their recruitment to target sites by promoting their adhesion to the vessel walls.

MMPs forms a family of protease sharing a conserved pro-domain, maintaining them in latent forms that can be activated under different circumstances [41,42]. Their roles within the organism comprise a wide range of functions from tissue homeostasis and remodeling to host defense. For example, extracellular matrix degradation by MMPs helps leukocytes to migrate to the inflame site [28]. In addition to this, MMP secretion is also a major contributor to tissue damage caused by inflammation. Deregulation of MMP expressions have been linked to many inflammatory and autoimmune diseases such as RA and inflammatory bowel diseases, diseases in which IL-21 is known to be involved [21,43–46]. Interestingly, IL-21 was previously reported to induce MMP secretion by intestinal fibroblasts and epithelial cells that can also be involved in inflammatory diseases [21,22]. Herein, we show that IL-21 induces MMP-9 secretion and gelatinase activity by HMDM but not by THP-1 cells and primary monocytes. Considering that MMPs, macrophages and IL-21 are all involved in RA, it is tempting to speculate that the direct effect of IL-21 on MMP secretion by macrophages and fibroblasts is a major contributor to the joint destruction observed in RA. Overall, our study support the importance to pursue investigating the IL-21 modulatory activity on the biology of cells of myeloid origin in order to limit undesired side effects of future therapeutic strategies targeting the IL-21/IL-21R system. For example, it would be interesting to study in parallel the biological activity of IL-21 on M1 and M2 polarized macrophages.

IL-21 can induce the migration and invasion of fibroblast-like synoviocytes isolated from RA patients [20]. The role of IL-21 in the recruitment of mononuclear phagocytes to the inflammatory site is still unclear. We have previously shown that administration of IL-21 into murine air pouches recruited an increased number and proportion of neutrophils and mononuclear cells in the exudates [9]. Because IL-21 is not chemotactic for monocytes (Fig. 1), it is tempting to speculate that the increased recruitment of mononuclear cells we previously reported was not induced by a direct effect of IL-21 on monocytes but rather by an indirect mechanism. Such an indirect mechanism could be an increase cytokine secretion from the murine air pouch lining cells. To support this, we previously documented that IL-4, one other γc user cytokine, was found to attract leukocytes (mainly neutrophil (60%) and monocytic (40%) cell populations) 9 h after its administration into air pouches [47]. Interestingly, we found that CCL-2 (also referred to as monocyte chemoattractant protein-1 or MCP-1) was the chemokine predominantly detected in the exudates in response to IL-4 before the arrival of leukocytes. After harvesting air pouch resident lining cells and incubated them in vitro with IL-4, we found that CCL-2 production was increased by IL-4 suggesting that IL-4 attracts leukocytes *in vivo* by an indirect mechanism involving the production of several analytes by, at least, resident cells.

Because we found previously that IL-21 can attract neutrophil and monocytic cells and that in contrast to LPS, IL-21 did not increase then local production of different cytokines/chemokines, including IL-6 and MIP-1α into murine air pouches, we were interested in investigating the possibility that IL-21 could induce the production of such soluble factors by human macrophages. Here, we reported that IL-21 did not increase by itself the production of IL-6, MIP-1α and GROα by macrophages and did not further increase the production of these factors known to be induced by LPS [48]. This further supports that IL-21 induces leukocyte infiltration *in vivo* by a mechanism distinct than that of LPS.

5. Conclusions

We reported herein that IL-21 possesses other biological activities in monocytic cells other than its ability to increase phagocytosis. It can increase the ability of monocytes to adhere onto a cell substratum such as the EA.hy926 endothelial cells by a mechanism not involving an increase cell surface expression of CD18, CD29, CD49D, CD11a, CD11b, ICAM-1, ICAM-3 and CD62L by requiring Syk activation. IL-21 was also found to increase the release of MMP-9 into the supernatants of HMDM as well as to induce gelatinase activity but do not appear to be an important inducer of cytokines/chemokines in these cells.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported partially by grants from Natural Sciences and Engineering Research Council of Canada [grant number RGPIN-2015-04894] and by the Institut de recherche Robert-Sauvé en santé et en sécurité du travail [grant number 2012-0053].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2019.02.002.

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