# Heterogeneity of adherent and non-adherent JAWS II dendritic cells

MARTA MARUSZEWSKA-CHERUIYOT[1], MAJA MACHCIŃSKA[1], MAGDALENA KIERASIŃSKA[2] and KATARZYNA DONSKOW-ŁYSONIEWSKA[1]

[1]Department of Experimental Immunotherapy, Faculty of Medicine, Lazarski University, 02-662 Warsaw; [2]Laboratory of Parasitology, General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, 01-163 Warsaw, Poland

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**Abstract.** Dendritic cells (DCs) are crucial in the development of immune responses. DC JAWS II is a murine cell line frequently used in DC studies. These cells are grown in two cell fractions: Adherent and non-adherent. The present study aimed to compare these two fractions in both immature and lipopolysaccharide (LPS)-activated JAWS II cells. The present study analysed the condition, phenotype, antigen uptake capability, signalling properties and the influence on the activity of T cells using flow cytometry, mixed cell reaction and ELISA methods. Adherent immature JAWS II cells exhibited increased endocytosis and decreased activation of the Pi3K signalling pathway. After LPS activation, adherent JAWS II cells exhibited increased expression levels of CD80 and CD86 costimulatory molecules, increased endocytosis and an elevated ability to induce T cell proliferation, compared with non-adherent cells. These results demonstrated that the two fractions of JAWS II adherent and non-adherent cells exhibited different properties and this should be taken into account in the planning of research.

## Introduction

Dendritic cells (DCs) are a major group of antigen presenting cells (APC). They play a crucial role in the development of anti-pathogen, anti-cancer and tolerogenic immune responses. DCs have potential in the treatment of autoimmune diseases and cancer. One of promising tools to investigate dendritic cells biology are DC lines such as JAWS II. JAWS II cells are immature DCs isolated from p53-deficient (p53-/-) C57BL/6 mouse bone marrow cells. Using murine cell lines is very useful due to high cell yields which facilitate experiments and allow repeated measurements. JAWS II cells are being used successfully in DC research on cancer vaccine delivery (1), anti-tumour effect (2), immunoparasitological study (3,4) and others (5-11). Immunotherapeutic vaccines show a promising weapon in the fight against cancer, especially when a personalized approach to the patient's disease is required. DC immunotherapy is very intensively researched as a form of cancer treatment (12,13). DC-based immunotherapy is created on functional activity of APC to develop an effective immune response against cancer with participation of T cells. In clinical studies, individuals with diseases like melanoma, breast cancer, prostate cancer, leukemia, and others are treated using DC cells-based vaccines (14).

The induction of adequate immunological responses is mostly dependent on the activation status of DCs. Immature, semi-mature and fully mature cells show different phenotypes and functions. DCs can induce tolerogenic or immunogenic response (15). In cancer immunotherapy, mature DCs with high ability to activate T cells with cytotoxic activity are expected. Maturation of dendritic cells results in increased expression of costimulatory e.g. CD40, CD80, CD86 and MHC molecules, decreased antigen internalization, changes in morphology of cells, as well as secretion of cytokines and chemokines. They drive T cell differentiation (16). Activation and maturation of dendritic cells can be induced by proinflammatory factors derived from bacteria e.g. lipopolysaccharide (LPS). Dendritic cells are still mysterious and can cause experimental difficulties. DC cultivation forms two fractions-non-adherent and adherent. Adherence as well as activation status can influence cell potential and properties. Yet some research conducted on dendritic cell lines does not describe which fractions of cells have been used. This information can be crucial in the interpretation of results, especially as human non-adherent and adherent monocyte-derived dendritic cells (mo-DCs) show differences in purity, surface markers expression, ability to process antigen and to stimulate T cells (17). Similarly,

*Correspondence to:* Dr Marta Maruszewska-Cheruiyot, Department of Experimental Immunotherapy, Faculty of Medicine, Lazarski University, 43 Świeradowska Street, 02-662 Warsaw, Poland
E-mail: m.maruszewska-cheruiyot@lazarski.pl

*Abbreviations:* BMDC, bone marrow-derived dendritic cells; DCs, dendritic cells; FITC-DX, isothiocyanate-labelled dextran; GM-CSF, granulocyte macrophage-colony stimulating factor; MFI, mean fluorescence intensity; MHC II, major histocompatibility complex class II; MLR, mixed lymphocyte reaction; Mo-DCs, monocyte-derived dendritic cells; RBC, red blood cells; TLR, toll like receptor

*Key words:* DCs, JAWS II, lipopolysaccharide, adherent, non-adherent

adherent and non-adherent murine bone marrow-derived dendritic cells differ (18).

Here, for the first time we are comparing non-adherent and adherent cell fractions of the DC line JAWS II. The study's objectives is to define their characteristics and differences which is important in the interpretation of the results of experimental studies on immunotherapy. We determined the condition, phenotype, antigen uptake capability, signalling properties and the influence on the activity of T cells of non-adherent and adherent cell fractions of JAWS II. To determine the properties of these cells in immature (non-activated) and activated status, we used LPS as a common DC stimulant.

## Materials and methods

*JAWS II cells culture*. Immature JAWS II cells isolated from p53-deficient ($p53^{-/-}$) C57BL/6 murine bone marrow cells were purchased from the American Type Culture Collection. JAWS II cells were then cultured in humidified atmosphere at 37ºC and 5% $CO_2$ in RPMI-1640 medium with L-Glutamine, 20% inactivated FBS, penicillin (100 U/ml), streptomycin (100 $\mu$g/ml) (Biowest) and 5 ng/ml GM-CSF (PeproTech). The cells were passaged twice a week. JAWS II cells were cultured at $5x10^5$/ml in 75 $cm^3$ bottles or 24-well plates for 24 h. The cells were then activated with 2 $\mu$g/ml LPS from Escherichia coli 055:B5 (Sigma-Aldrich; Merck KGaA) for 48 h. The cells were divided into two fractions: non-adherent-cells suspended in the medium and adherent cells-adjacent to the substrate. The experiment examined four groups of cells: immature (non-activated) adherent and non-adherent JAWS II, as well as LPS-activated adherent and non-adherent cells. The non-adherent cells were collected and adherent cells removed with non-enzymatic cell detachment solution. After 48 h of culture, the cells were collected and washed twice in ice-cold PBS for analysis on a Muse Cell Analyzer (Merck Millipore). The Muse Count and Viability Kit provide rapid and reliable determinations of viability and total cell count. The ratio was calculated by dividing the number of non-adherent cells by the number of adherent cells. Apoptosis was examined with the Muse Annexin V & Dead Cell Assay. Phosphoinositide-3-kinase (Pi3K) and mitogen-activated protein kinase (MAPK) signal pathway activation was analysed with the Muse PI3K/MAPK Dual Pathway Activation Kit based on the percentage of Akt and ERK1/2 activated cells. All procedures were performed according to the manufacturer's guidelines.

*Phenotypic characterisation by flow cytometry*. JAWS II cells were characterised by flow cytometry. The following fluorochrome-conjugated monoclonal antibodies were used according to the manufacturer's protocols: CD11c-SB436 (clone N418; eBioscience), CD40-SB600 (clone 1310; eBioscience), CD80-APC (clone 16-10A1, eBioscience), CD86-eFluor780 (clone GL1; eBioscience) and MHC II-FITC (clone NIMR-4; eBioscience). Fixable Viability Dye (FVD) eFluor 455UV (eBioscience) was used as a vital dye to exclude dead cells. Briefly, cells were collected, washed and resuspended in PBS (pH 7.2). Next, the cells ($1x10^6$) were incubated with appropriate monoclonal antibodies for 30 min in the dark at 4˚C. Following this, the cells were washed twice with Cell Wash (Becton-Dickinson) containing 0.5% bovine serum albumin (BSA, Biowest) and acquired on a CytoFLEX LX (Beckman Coulter), calibrated daily using CytoFLEX Daily QC Fluorospheres (Beckman Coulter). Compensation settings were conducted using single-stained cells or the VersaComp Antibody Capture Bead Kit (Beckman Coulter). The following gating strategy was used: A time gate was initially applied to exclude any electronic noise and artifact. Next, based on size and granularity, JAWS II cells were gated in a forward scatter area (FSC-A) vs. side scatter area (SSC-A). Then, doublet cells were excluded using FSC-A/FSC-height (FSC-H), FSC-A/FSC-Width and SSC-A/SSC-height (FSC-H) parameters. Within the singlet cell population, viable JAWS II were gated based on the dim expression of the Fixable Viability Dye (FVD), followed by expression of examined markers: CD80, CD86, MHCII or CD40. In all the experiments at least 100,000 events were analysed for each sample. Positive staining and the gating strategy was determined by the comparison to the unstained control and the fluorescence minus one (FMO) controls. Data was analysed using Kaluza Analysis Software version 2.1 (Beckman Coulter). The results were shown as the percentage of positively labelled cells and the mean fluorescence intensity (MFI) calculated by CytoFLEX LX.

*Endocytosis assay*. To determinate the ability of cells to take up, fluorescein isothiocyanate-conjugated dextran (FITC-DX 70,000, Sigma-Aldrich; Merck KGaA) was used. The cells ($1x10^6$) were incubated with FITC-DX (2 mg/ml) for 50 min at 37˚C (control plate at 4˚C). At the end of the incubation, the cells were collected and washed three times by centrifugation at 4˚C in PBS (5 min, 300 x g) and re-suspended in 0.1 ml ice-cold PBS (pH 7.2) with 0.5% BSA for flow cytometry analysis.

*T cell isolation and mixed lymphocyte reaction (MLR)*. Male C57BL/6 mice were euthanized by cervical dislocation and their spleens isolated aseptically (n=8). The spleens were pressed through a nylon cell strainer (BD Falcon) to produce a single-cell suspension. Erythrocytes in the cell suspension were depleted with red blood cell (RBC) lysis buffer. Lymphocytes were purified using a MagniSort Mouse T cell Enrichment Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The purity was determined by flow cytometry (CytoFlex LX, Beckman Coulter) using the CD3-eFluor506 monoclonal antibody (clone 17A2; eBioscience) post isolation. The T cells were then stained with Cell Proliferation Dye eFluor 670 (CPD; eBioscience) and $1x10^6$ T cells cocultured with $1x10^5$ adherent or non-adherent JAWS II cells (ratio 10:1) in 24-well plates for 120 h in the culture medium described above (37˚C, 5% $CO_2$). After culturing, the supernatants were collected for enzyme-linked immunosorbent assay (ELISA), then the cells were harvested and labelled with CD3-eFluor506 monoclonal antibody (clone 17A2; eBioscience) in order to identify T cells. All procedures were performed according to the manufacturer's protocols. The cells were prepared for flow cytometry analysis and analysed as described above.

*Cytokine detection in cell culture*. An ELISA method was used to determine IL-6, IL-10, IL-17A, IFN-γ and TGF-β1 cytokine levels in cell-free supernatants according to the manufacturer's guidelines (e-Biosciences, Thermo Fisher Scientific). For the

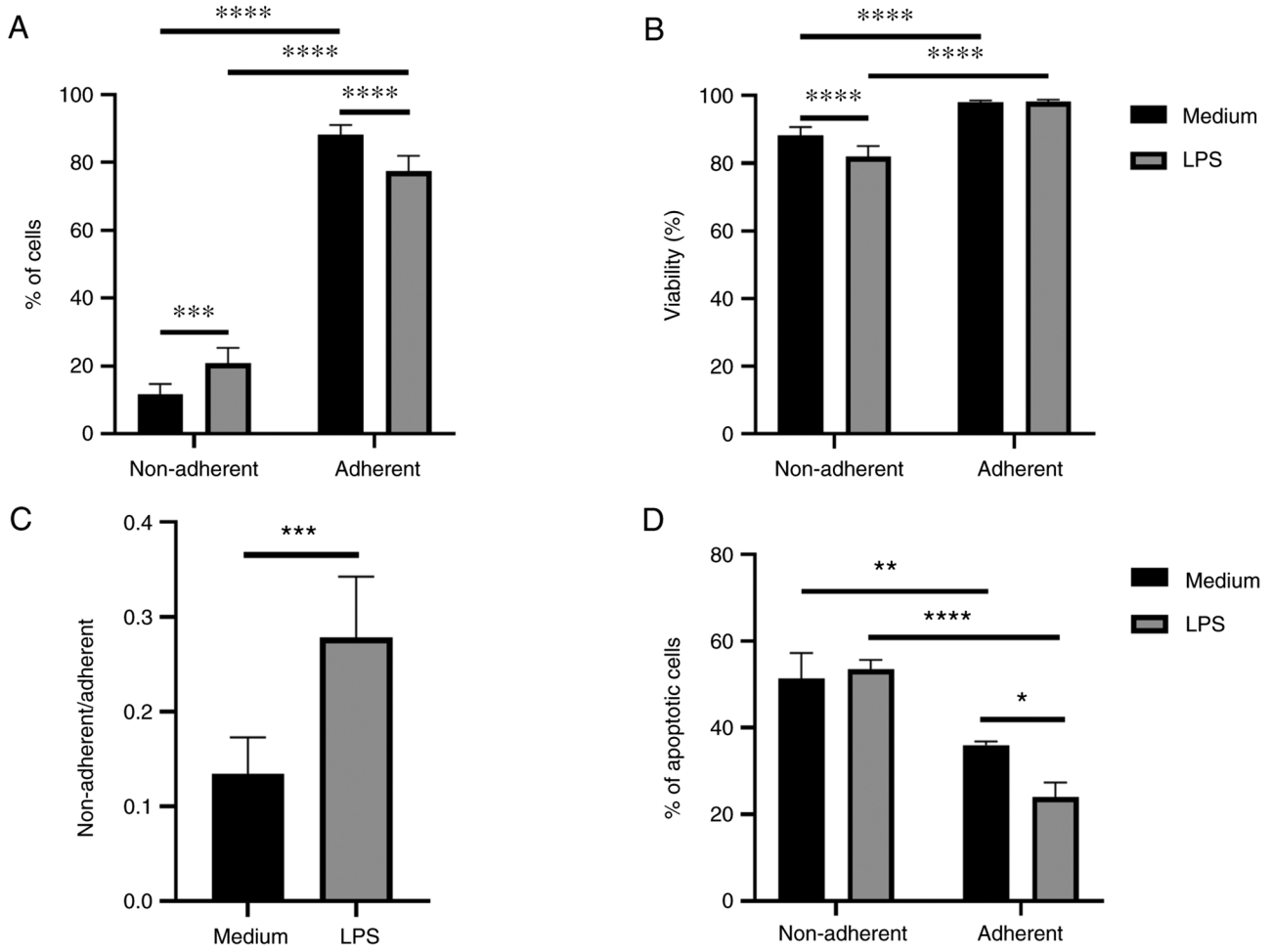


Figure 1. Percentage, viability, ratio and apoptosis of adherent and non-adherent JAWS II cells. JAWS II cells were activated with LPS (2 $\mu$g/ml). (A) The number of non-adherent and adherent cells and (B) their viability were evaluated using the Muse Count and Viability Kit (Muse Cell Analyzer). The data are presented as percentage of live cells ± SD. (C) The ratio was calculated by dividing the number of non-adherent cells by the number of adherent cells. (D) Apoptosis was evaluated using The Muse Annexin V and Dead Cell Assay and presented as the percentage of late apoptotic/dead cells ± SD, N=6. The lines indicate significant differences. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 and $^{****}$P<0.0001. LPS, lipopolysaccharide.

TGF-β1 measurement, the samples were acidified, and the latent and active cytokine excreted into the culture medium measured in each sample. The colorimetric reaction was determined at 450 nm using the Synergy™ H1 Microplate Reader (BioTek). The mean optical densities (OD) of the triplicate cultures were compared with the standard curves prepared using recombinant cytokines.

*Statistical analysis*. The significance of the differences between two groups was determined by the Student's unpaired, two-tailed t-test. The two-way analysis of variance (ANOVA) was used for multiple group comparisons (GraphPad Software Inc.). The ANOVA was followed by Tukey's post hoc analyses. Data were expressed as mean ± SD. A P-value of <0.05 was considered to be statistically significant.

## Results

*Viability of adherent and non-adherent JAWS II cells*. The percentage of cells, viability, and the percentage of apoptotic cells were investigated in both fractions. The ratio of the JAWS II cell fractions was calculated as non-adherent/adherent cells. There were more adherent than non-adherent cells in both, immature and LPS-activated JAWS II cell culture. Activation with LPS increased percentage of non-adherent cells and decreased percentage of cells in adherent fraction (Fig. 1A). Higher viability of cells was observed in adherent fraction of immature and LPS activated cells. A lower viability of cells was observed in the non-adherent fraction after LPS treatment (Fig. 1B). The ratio of JAWS II cell fractions was significantly higher after LPS activation compared to non-activated cells (Fig. 1C). The percentage of apoptotic cells in the adherent fraction was decreased in comparison to non-adherent. Apoptosis of adherent JAWS II cells was even lower after activation with LPS and significantly reduced compared to non-adherent LPS-activated cells (Fig. 1D).

*Differential expression of surface markers by adherent and non-adherent cells*. Adherent and non-adherent cells were phenotyped by flow cytometry. Immature non-adherent and adherent JAWS II cells were not significantly phenotypically different. In LPS activated, both adherent and non-adherent cells had increased MFI values of the costimulatory molecules CD80 and CD86, compared with immature cells

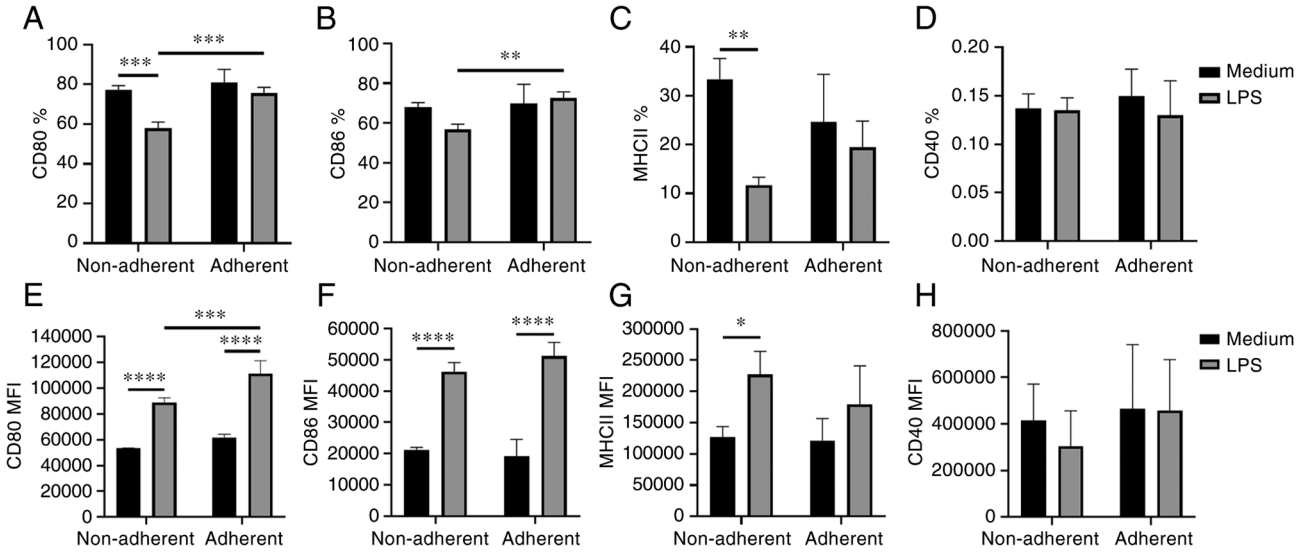


Figure 2. Phenotype of adherent and non-adherent JAWS II cells. Surface marker expression was evaluated by flow cytometry after 48-h stimulation of JAWS II cells with LPS (2 $\mu$g/ml). The graphs show the percentage of (A) CD80, (B) CD86, (C) MHCII, (D) CD40 positive cells, and MFI of (E) CD80, (F) CD86, (G) MHCII and (H) CD40 ± SD, N=4. The lines indicate significant differences. $^{*}$P<0.05, $^{**}$P<0.01, $^{***}$P<0.001 and $^{****}$P<0.0001. LPS, lipopolysaccharide; MFI, mean fluorescence intensity; MHC, major histocompatibility complex.

(Fig. 2E and F). What is more, higher percentage of CD80$^{+}$ and CD86$^{+}$ cells, as well as higher CD80 MFI values were observed in LPS-activated adherent cells compared to the LPS-activated non-adherent cells (Fig. 2A, B and E). The lower percentage of MHC II$^{+}$ cells but higher MFI of MHC II was observed in the LPS-activated non-adherent cells compared to these cells without LPS stimulation (Fig. 2C and G). No significant changes were observed in the percentage of CD40$^{+}$ cells and the MFI of CD40 (Fig. 2D and H). Also, there were no significant differences in the expression of CD11c (data not shown).

*Immature adherent and non-adherent JAWS II cells exhibit different endocytosis and signalling properties*. To determine the endocytic activity of JAWS II cells, which is crucial in antigen presentation, cellular FITC-dextran uptake was measured by flow cytometry. FITC-dextran uptake was higher in adherent cells compared to non-adherent cells in both, immature and LPS-activated JAWS II cells (Fig. 3A and B). To evaluate signalling in both fractions of JAWS II cells, the MAPK and Pi3K signal pathways were examined. The immature, non-adherent fraction showed an increased percentage of pAKT positive cells in comparison with adherent cells. Activation with LPS did not result in significant changes in the Pi3K signal pathway (Fig. 3C). No significant changes were observed in MAPK activation based on the percentage of pERK1/2 positive cells (data not shown).

*LPS-activated adherent and non-adherent JAWS II cells differentially influence T cell activity*. In order to evaluate how non-adherent and adherent JAWS II cells impact T cells functions, proliferation and cytokine production were investigated. Unstimulated T cells cultured without JAWS II cells were used as the negative control, and T cells stimulated with CD3 and CD28 antibodies were treated as the positive control. LPS-activated, adherent JAWS II cells induced stronger proliferation of T cells than non-adherent LPS-activated JAWS II cells. A similar effect, but not statistically significant, was observed for immature cells (Fig. 4). LPS-activated non-adherent JAWS II induced significantly higher level of IL-6 and TGF-β1 than did immature non-adherent cells. Stimulation of non-adherent cells with LPS resulted in elevated production of IL-6 and TGF-β1 in comparison to adherent cells (Fig. 5A and B). IL-17A was produced in small amounts by non-adherent cells both treated and not treated with LPS, with no detected production by adherent JAWS II cells (Fig. 5C). No significant changes in IL-10 production were observed (Fig. 5D). IFN-γ production was not observed (data not shown).

## Discussion

In the present study, we evaluated the viability, phenotype and activity of immature and LPS-activated JAWS II cells. The cells were divided into two fractions: non-adherent (cells suspended in the medium) and adherent cells (adjacent to the substrate). The results indicated that these two fractions of JAWS II cells differs in various ways.

At the beginning we evaluated JAWS II cells condition. There were more adherent cells than non-adherent cells and the adherent cells had better viability. However, activation with LPS resulted in a larger proportion of non-adherent cells in comparison with unstimulated cells. Next step was to examine the surface phenotype. The expression of markers associated with T cell activation (CD80, CD86, MHC II and CD40) was determined. Immature non-adherent and adherent JAWS II cells were not significantly phenotypically different. This is in line with previous outcomes on JAWS II cells (1) and human monocyte-derived dendritic cells (mo-DCs), where only the percentage of CD86 positive cells was statistically different (17).

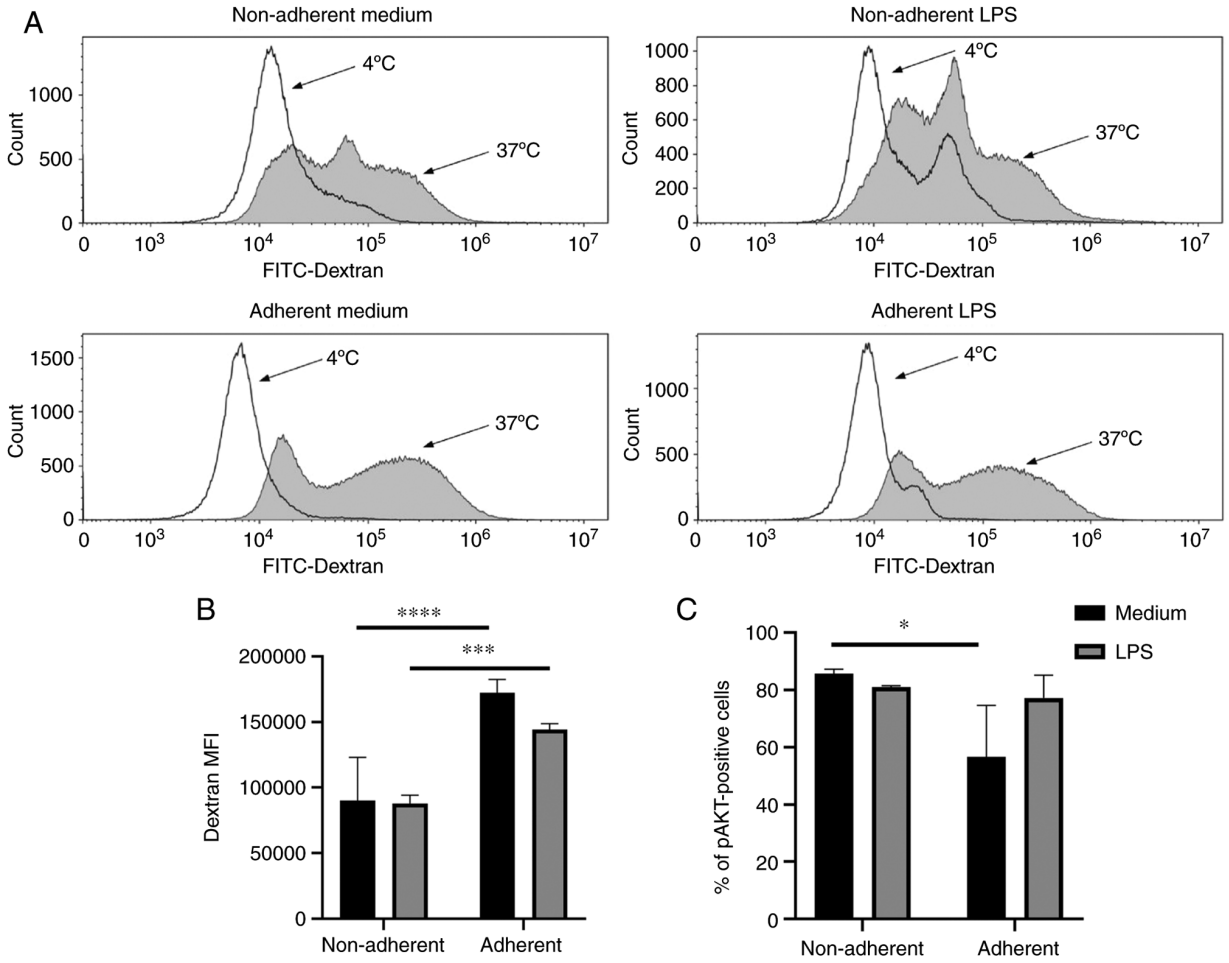


Figure 3. Activity of adherent and non-adherent JAWS II cells. JAWS II cells were stimulated with LPS for 48 h (2 $\mu$g/ml) and incubated for 50 min at 37˚C with FITC-DX. Cells incubated at 4˚C with FITC-DX represented the background signal. Endocytosis was evaluated by flow cytometry. Data are shown as (A) representative histograms of FITC-DX uptake at both 37 and 4˚C (control) and (B) as MFI of FITC-DX (MFI 37˚C-MFI 4˚C) ± SD. (C) Pi3K signal pathway activation was analysed with the Muse PI3K/MAPK Dual Pathway Activation Kit with Muse Analyzer. Data are presented as the percentage of Akt activated cells ± SD, N=3. The lines indicate significant differences. $^{*}$P<0.05, $^{***}$P<0.001 and $^{****}$P<0.0001. DX, dextran; LPS, lipopolysaccharide; MFI, mean fluorescence intensity; pAKT, phosphorylated AKT.

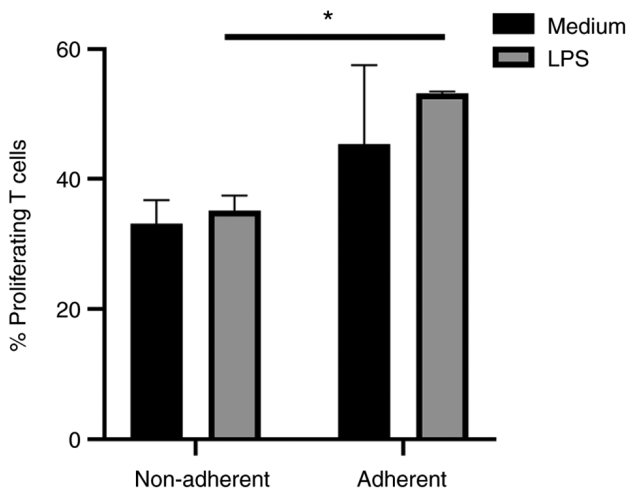


Figure 4. Influence of non-adherent and adherent JAWS II cells on T cells. JAWS II cells were stimulated with LPS for 48 h (2 $\mu$g/ml) and then added to T cells isolated from spleen, and stained with CPD. JAWS II and T cells were cultured in 1:10 ratio. The cells were collected after 120 h. Proliferation potential is presented as the mean percentage of T cells with reduced level of CPD evaluated by flow cytometry ± SD, N=3. The lines indicate significant differences. $^{*}$P<0.05. CPD, Cell Proliferation Dye; LPS, lipopolysaccharide.

However, we observed differences in the phenotype between the two fractions of LPS-activated JAWS II cells. In LPS-activated JAWS II cells a higher percentages of CD80- and CD86-positive cells were observed in the adherent fraction. Also, the MFI of CD80 was increased in LPS-activated adherent JAWS II cells. These results suggest, that LPS-activated adherent JAWS II cells may be more effective in antigen presentation and T cells activation than LPS-activated non-adherent JAWS II cells.

LPS activation of JAWS II resulted in decreased percentage of CD80- and MHC II-positive cells, but it was associated with higher expression (MFI) of CD80, CD86, and MHC II molecules in non-adherent cells. A reduced percentage of cells can result from several issues, such as the condition of cells in the LPS environment. However, the marker expression on the cell surface may be sufficient for effective T cell activation. We have obtained similar results in our previous work concerning human monocyte-derived DCs (mo-DCs). After LPS activation, only the MFI values were significantly higher compared to immature DCs for all examined receptors: MHC II, CD40, CD80, CD83, CD86, CCR7, TLR2, TLR4 (19).

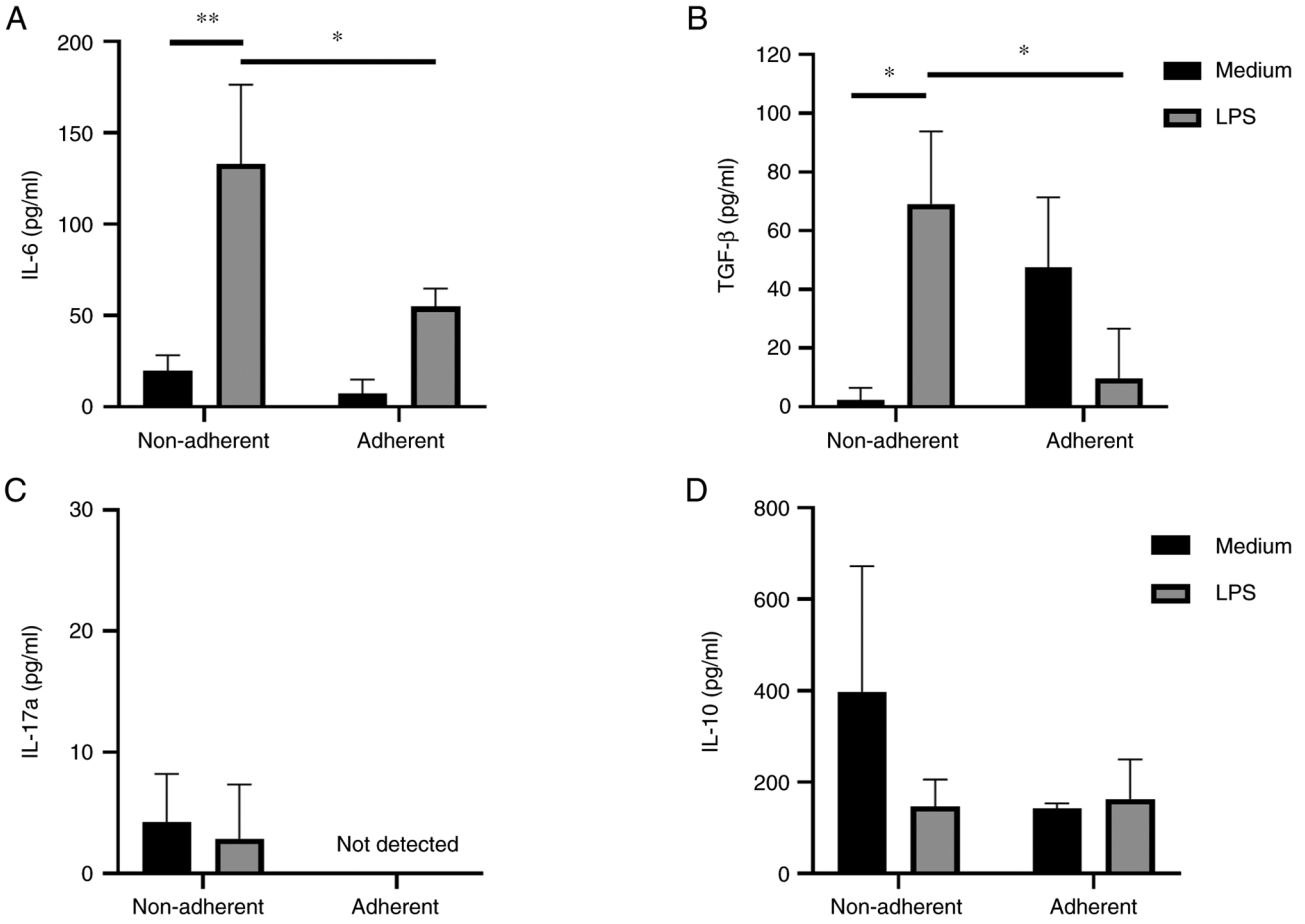


Figure 5. Cytokine production in the coculture of T cells with non-adherent and adherent JAWS II cells. JAWS II cells were stimulated with LPS for 48 h (2 $\mu$g/ml) and then added to T cells isolated from spleen, and stained with CPD. JAWS II and T cells were cultured in 1:10 ratio. The supernatants were collected after 120 h. Levels of (A) IL-6, (B) TGF-β1, (C) IL-17A and (D) IL-10 were measured by ELISA. Data are presented as the mean cytokine concentration ± SD (pg/ml), N=3. The lines indicate significant differences. $^{*}$P<0.05, $^{**}$P<0.01. CPD, Cell Proliferation Dye; LPS, lipopolysaccharide.

With the maturation of DCs, the ability to uptake antigen decreases. Our studies confirmed that after stimulation with LPS, cells showed reduced endocytosis, but not statistically significant. We have observed, that adherent JAWS II cells exhibited significantly higher endocytosis compared tonon-adherent cells. This may suggest that adherent cells demonstrate increased endocytic capacity. Yi and Lu (17) reported that mature human mo-DCs, adherent cells show higher antigen uptake than non-adherent cells. We have obtained similar results.

Our findings demonstrate that there were decreased numbers of immature adherent cells with activated Pi3K signalling compared to non-adherent cells. Pi3K signalling plays an important role in various cellular processes such as proliferation, migration or antigen presentation. In addition, Pi3K signalling is involved in the pathogenesis of autoimmune diseases and immunity against cancer (20,21). The inhibition of Akt phosphorylation in bone-marrow derived DCs (BMDC) by IL-10 suppresses IKK/NF-κB activation (22,23).

The crucial role of DCs is antigen presentation and T cells activation, hence we evaluated proliferation and cytokine production of T cells isolated from murine spleen with adherent and non-adherent JAWS II cells. We observed increased proliferation of T cells cocultured with LPS-activated, adherent DCs in line with decreased production of IL-6, and TGF-β1, compared to non-adherent LPS-activated cells. These results confirmed, that LPS-activated adherent JAWS II cells may induce T cell proliferation. The experiment with human DCs also showed that adherent cells more effectively induced proliferation of T cells than non-adherent DC (17). However, Wang *et al* (18) showed that non-adherent bone-marrow derived DCs (BMDC) more effectively stimulated proliferation of CD4 T cells isolated from lymph nodes than adherent cells. The difference in the results could be a consequence of the source of T cells.

To summarise, adherent immature JAWS II cells show increased endocytosis and decreased activation of the Pi3K signal pathway. They also induced increased production of TGF-β1 by T cells in comparison to non-adherent cells. On the other hand, adherent, LPS-activated JAWS II cells showed increased expression of CD80 and CD86 costimulatory molecules, increased endocytosis and an elevated ability to induce proliferation of T cells in MLR with decreased production of IL-6, IL17A and TGF-β1 compared to non-adherent LPS-activated cells.

Choosing the right fraction for cancer immunotherapy research can be crucial. In immunotherapy of cancer, mature DCs with great capacity to activate T cells with cytotoxic activity are expected. Based on our results, LPS-activated adherent JAWS II cells fraction appears to be more favorable for future DC-vaccine evaluation against cancers. Such cells showed increased expression of costimulatory molecules and

induced T cells proliferation and may be more effective in T cell activation.

Non-adherent fraction shows some properties characteristics for tolerogenic DCs. Tolerogenic DCs are immature dendritic cells, induce immune tolerance and are able to inhibit T cell response. Decreased CD80, CD86 and MHC II expression, lower percentage of proliferating T cells, increased IL-10 production and activation of the Pi3K signal pathway may indicate tolerogenic functions of non-adherent immature JAWS II compared to adherent (24,25). Some results show that DC after longer exposition to LPS can become more 'tolerant' or 'exhausted'. This is associated with the production of Th2 cytokines instead of the development of a pro-inflammatory Th1 immune response (26). Other studies indicate that DC after long exposure to LPS wait for a signal from T cells, necessary for their further activity (27).

Heterogenicity of dendritic cells has been discussed for a long time; however, this problem is not flawlessly described. Choosing adherent or non-adherent cells is not only relevant for DCs, but also for other types of cells with potential use in clinical studies (28). More attention should be paid to which fraction of DCs is chosen. The effect of two mixed fractions can be abolished. In addition, a lack of indication of the used fraction can result in apparently different results. We showed that the two fractions of JAWS II, adherent and non-adherent cells, show different properties. In addition, differences in the phenotype and activity of adherent and non-adherent cells are more significant after LPS activation. Therefore, both the adherence and activation status of DCs are important when planning experiments.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

MMC, MM, MK and KDL contributed to the study conception and design. MMC, MM and KDŁ confirm the authenticity of all the raw data. MMC performed the experiments, analysed data and wrote the manuscript. MM performed the experiments, analysed data and corrected the manuscript. MK performed the experiments and analysed data. KDŁ analysed the data and corrected the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

All methods involving animal studies are in line with ARRIVE EU guidelines Directive 2010/63/EU on animal experimentation.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Vang KB, Safina I, Darrigues E, Nedosekin D, Nima ZA, Majeed W, Watanabe F, Kannarpady G, Kore RA, Casciano D, *et al*: Modifying dendritic cell activation with plasmonic nano vectors. Sci Rep 7: 5513, 2017.
2. Zapala L, Drela N, Bil J, Nowis D, Basak GW and Lasek W: Optimization of activation requirements of immature mouse dendritic JAWSII cells for in vivo application. Oncol Rep 25: 831-840, 2011.
3. Jittimanee S, Wongratanacheewin S, Kaewraemruaen C and Jittimanee J: Opisthorchis viverrini antigens up-regulates the expression of CD80 and MHC class II in JAWSII mouse dendritic cells and promotes IL-10 and TGF-β secretions. Parasitol Int 84: 102401, 2021.
4. Maruszewska-Cheruiyot M, Donskow-Łysoniewska K, Piechna K, Krawczak K and Doligalska M: L4 stage Heligmosomoides polygyrus prevents the maturation of dendritic JAWS II cells. Exp Parasitol 196: 12-21, 2019.
5. Bieńkowska A, Kiernozek E, Kozlowska E, Zarzycki M and Drela N: Thymus-deriving natural regulatory T cell generation in vitro: Role of the source of activation signals. Immunol Lett 162: 199-209, 2014.
6. Eko FO, Mania-Pramanik J, Pais R, Pan Q, Okenu DM, Johnson A, Ibegbu C, He C, He Q, Russell R, *et al*: Vibrio cholerae ghosts (VCG) exert immunomodulatory effect on dendritic cells for enhanced antigen presentation and induction of protective immunity. BMC Immunol 15: 584, 2014.
7. Egger M, Jürets A, Wallner M, Briza P, Ruzek S, Hainzl S, Pichler U, Kitzmüller C, Bohle B, Huber CG and Ferreira F: Assessing protein immunogenicity with a dendritic cell line-derived endolysosomal degradome. PLoS One 6: e17278, 2011.
8. Hu Y, Li M, Lu B, Wang X, Chen C and Zhang M: Corticotropin-releasing factor augments LPS-induced immune/inflammatory responses in JAWSII cells. Immunol Res 64: 540-547, 2016.
9. Mutlu EC, Kaya Ö, Wood M, Mager I, Topkara KÇ, Çamsarı Ç, Birinci Yildirim A, Çetinkaya A, Acarel D and Odabaşı Bağcı J: Efficient doxorubicin loading to isolated dexosomes of immature JAWSII cells: Formulated and characterized as the bionanomaterial. Materials (Basel) 13: 3344, 2020.
10. Safina I, Alghazali KM, Childress L, Griffin C, Hashoosh A, Kannarpady G, Watanabe F, Bourdo SE, Dings RPM, Biris AS and Vang KB: Dendritic cell biocompatibility of ether-based urethane films. J Appl Toxicol 41: 1456-1466, 2021.
11. Ssemakalu CC, Ubomba-Jaswa E, Motaung KSCM and Pillay M: Solar inactivated Vibrio cholerae induces maturation of JAWS II dendritic cell line in vitro. J Water Health 18: 494-504, 2020.
12. Baldin AV, Savvateeva LV, Bazhin AV and Zamyatnin AA Jr: Dendritic Cells in anticancer vaccination: Rationale for ex vivo loading or in vivo targeting. Cancers (Basel) 12: 590, 2020.
13. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF and Sancho D: Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol 20: 7-24, 2020.
14. Salah A, Wang H, Li Y, Ji M, Ou WB, Qi N and Wu Y: Insights into dendritic cells in cancer immunotherapy: From bench to clinical applications. Front Cell Dev Biol 9: 686544, 2021.
15. Lutz MB and Schuler G: Immature, semi-mature and fully mature dendritic cells: Which signals induce tolerance or immunity? Trends Immunol 23: 445-449, 2002.
16. Dalod M, Chelbi R, Malissen B and Lawrence T: Dendritic cell maturation: Functional specialization through signaling specificity and transcriptional programming. EMBO J 33: 1104-1116, 2014.
17. Yi HJ and Lu GX: Adherent and non-adherent dendritic cells are equivalently qualified in GM-CSF, IL-4 and TNF-α culture system. Cell Immunol 277: 44-48, 2012.

18. Wang J, Dai X, Hsu C, Ming C, He Y, Zhang J, Wei L, Zhou P, Wang CY, Yang J and Gong N: Discrimination of the heterogeneity of bone marrow-derived dendritic cells. Mol Med Rep 16: 6787-6793, 2017.
19. Machcińska M, Kotur M, Jankowska A, Maruszewska-Cheruiyot M, Łaski A, Kotkowska Z, Bocian K and Korczak-Kowalska G: Cyclosporine A, in contrast to rapamycin, affects the ability of dendritic cells to induce immune tolerance mechanisms. Arch Immunol Ther Exp (Warsz) 69: 27, 2021.
20. Aksoy E, Saveanu L and Manoury B: The isoform selective roles of PI3Ks in dendritic cell biology and function. Front Immunol 9: 2574, 2018.
21. Datler H, Vogel A, Kerndl M, Baumgartinger C, Musiejovsky L, Makivic N, Frech S, Niederreiter B, Haider T, Pühringer M, *et al*: PI3K activity in dendritic cells exerts paradoxical effects during autoimmune inflammation. Mol Immunol 111: 32-42, 2019.
22. Bhattacharyya S, Sen P, Wallet M, Long B, Baldwin AS Jr and Tisch R: Immunoregulation of dendritic cells by IL-10 is mediated through suppression of the PI3K/Akt pathway and of IkappaB kinase activity. Blood 104: 1100-1109, 2004.
23. Cahill CM, Rogers JT and Walker WA: The role of phosphoinositide 3-kinase signaling in intestinal inflammation. J Signal Transduct 2012: 358476, 2012.
24. Hu J and Wan Y: Tolerogenic dendritic cells and their potential applications. Immunology 132: 307-314, 2011.
25. Svajger U and Rozman P: Tolerogenic dendritic cells: Molecular and cellular mechanisms in transplantation. J Leukoc Biol 95: 53-69, 2014.
26. Langenkamp A, Messi M, Lanzavecchia A and Sallusto F: Kinetics of dendritic cell activation: Impact on priming of TH1, TH2 and nonpolarized T cells. Nat Immunol 1: 311-316, 2000.
27. Abdi K, Singh NJ and Matzinger P: Lipopolysaccharide-activated dendritic cells: 'Exhausted' or alert and waiting? J Immunol 188: 5981-5989, 2012.
28. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM and Caplan AI: Mesenchymal stem cell perspective: Cell biology to clinical progress. NPJ Regen Med 4: 22, 2019.