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ORIGINAL ARTICLE

PMA induces the differentiation of monocytes into immunosuppressive MDSCs

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Abstract

The induction of immune tolerance without the use of immunosuppressive drugs is a crucial problem in organ transplantation. The use of myeloid-derived suppressor cells (MDSCs) as a cell-based adjuvant immunosuppressive therapy is a bright clinical prospect in organ transplantation. MDSCs with stable immunosuppressive activities can be used to treat immune-related diseases. In this study, macrophage colony-stimulating factor (M-CSF) was used to promote myeloid progenitor cell differentiation, and phorbol 12-myristate 13-acetate (PMA) was added to induce MDSCs at the later stage of induction *in vitro*. Cell phenotypes were detected by flow cytometry and mRNA was detected by real-time–polymerase chain reaction (RT–PCR). A mouse skin transplantation model was used to investigate the cell inhibitory function. The combination of PMA and M-CSF induced the differentiation of myeloid-derived monocytes into MDSCs. MDSCs were found to induce immune tolerance by inhibiting the proliferation and activation of T cells, promoting cytokine secretion and inducing T cell transformation to regulatory T cells (T_{reg}). PMA significantly up-regulated the expression of Arg-1 and the Arg-1 protein expression in MDSCs and arginase 1 (Arg-1) inhibitor nor-NOHA reversed the MDSC immunosuppressive activity, indicating the involvement of the Arg-1 pathway in MDSC-mediated immunosuppression. M-CSF + PMA-induced MDSCs also significantly prolonged the survival time of skin grafts in mice, showing that MDSCs exert immunosuppressive effects *in vivo*. We describe a novel scheme to induce immunosuppressive MDSCs *in vitro*. MDSCs induced by M-CSF with PMA showed stable immunosuppression. MDSCs induced by this protocol may benefit patients with organ transplantation through immune regulation.

KEYWORDS

immune tolerance, myeloid-derived suppressor cells, PMA, transplantation

Haozhou Wang, JiaWei Ji and Yuan Zhuang contributed equally to this work.

INTRODUCTION

Although immunosuppressive therapy after organ transplantation effectively prevents allograft rejection in the short term, the incidence of chronic rejection and long-term transplantation failure remains high. Long-term use of immunosuppressants can significantly reduce the systemic immune function, increasing the patient's vulnerability to fatal infections and malignant tumors [1]. In this context, cell therapy has emerged as a promising strategy to reduce the need for immunosuppressive drugs and ultimately induce long-term donor-specific tolerance [2]. Regulatory myeloid cells, such as tolerogenic dendritic cells, macrophage suppressor cells and myeloid-derived suppressor cells, have received attention as they can both regulate T cells directly or induce the actions of regulatory T cells [3,4].

Myeloid-derived suppressor cells (MDSCs) are a subset of innate immune cells that can inhibit innate and acquired immunity [5,6]. In pathological conditions, such as cancer, infection, trauma, organ transplantation and some autoimmune diseases, normal myeloid cell differentiation is blocked and many myeloid cell precursors expand into heterogeneous MDSCs with inhibitory functions [7]. Because of this, MDSCs, unlike granulocytes, monocytes, macrophages or dendritic cells (DC), lack specific surface markers but have a combination of myeloid surface markers together with the morphological characteristics of granulocytes or monocytes. In mice, MDSCs are termed CD11b and Gr-1 cells. CD11b is a subunit of the β2 integrin macrophage-1 antigen (Mac-1) and is expressed on the surface of granulocytes, monocytes, macrophages and DC cells, while Gr-1 is mainly expressed on the surface of granulocytes and macrophages. MDSCs can be further classified according to the cell surface expression of lymphocyte antigen 6 (Ly6)C and Ly6G and nuclear morphology and divided into two subgroups: monocyte-like MDSCs (M-MDSCs) and granulocyte-like MDSCs (G-MDSCs). M-MDSCs express $CD11b^{+}Ly6G^{-}Ly6C^{high}$ and G-MDSCs express $CD11b^{+}Ly6G^{+}Ly6C^{low}$ [8,9]. MDSCs can inhibit both innate and adaptive immunity. They can directly inhibit the proliferation and differentiation of lymphocytes and promote the production of forkhead box protein 3 $(FoxP3)^{+}$ regulatory T lymphocytes (T_{reg} cells). In addition, MDSCs can also exert their immunosuppressive function by inhibiting natural killer (NK) cells and DC cells [10,11].

Arginase-1 (Arg-1) induces the expression of inducible nitric oxide synthase (iNOS) or indoleamine 2,3-dioxygenase 1 (IDO) to consume nutrients necessary for T cell activation or interferes with Janus kinase 3/signal transducer and activator of transcription 5 (JAK3/STAT-5) signal transduction in T cells through the promotion of nitric oxide (NO) secretion, the mechanism by which MDSCs suppress immune function [12,13]. MDSCs are thus closely involved in the induction and maintenance of immune tolerance. In many transplantation models, including islet [14], heart [15], skin [16] and kidney transplantation [17], MDSCs can reduce the incidence of rejection by inhibiting the immune response. Therefore, MDSCs offer great potential for the development of treatments to suppress immune rejection after organ transplantation [18,19].

Phorbol 12-myristate 13-acetate (PMA) is a protein kinase C (PKC) activator widely used in cell experiments *in vitro* that extensively influences cell metabolism, growth, differentiation and proliferation. PMA is easier to preserve and cheaper than cytokines. We first found that macrophage colony-stimulating factor (M-CSF) combined with PMA could produce MDSCs with high immunosuppressive activities that could be applied to immunomodulatory cell therapy.

MATERIALS AND METHODS

Mice

C57BL/6 (B6) mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (China). Six- to 10-year-old mice from the same litter were used in this study. All mice were reared in an aseptic environment in the medical research center of Beijing Chaoyang Hospital. All animal experiments were approved by the ethics committee of Beijing Chaoyang Hospital.

Induction of MDSCs *in vitro*

Bone marrow cells were prepared by flushing tibias and femurs of B6 mice with phosphate-buffered saline (PBS). Red blood cells were lysed with 0.2 and 1.6% stroke-physiological saline solution. Then cells were planted in 100-mm dishes (Corning, New York, USA) for 2 h and adherent cells were discarded to exclude bone marrow macrophage. For MDSC induction, 4×10^5/ml non-adherent bone marrow cells were cultured with 50 ng/ml M-CSF with 0.5 μg/ml PMA in RPMI-1640 medium supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml) and 10% heat inactivated fetal bovine serum (FBS) for 7 days at 37°C, 5% CO_2. PMA was added on day 6 and half the medium was changed on days 3 and 5.

T cell proliferation assay

Single-cell suspension was prepared from spleens of B6. Cells were labeled with 2 μM carboxyfluorescein succinimidyl ester (CFSE) for 5 min in PBS at 37°C and washed twice with PBS. The labeled cells were then stimulated with concanavalin A (ConA) (2 μg/ml) in the presence of different doses of MDSCs as indicated for 72 h. The cell proliferation was then

determined by flow cytometry (FCM) after staining with anti-CD4 or anti-CD8 monoclonal antibody (mAb), respectively.

Cell staining and flow cytometry

For surface marker staining of MDSCs, induced cells at 5×10^5/tube in 100 μl phenylbenzimidazole sulfonic acid (PBSA) (0.1%) were incubated with the appropriate antibody at 4°C in the dark for 30 min. Then cells were washed with PBSA and analyzed by flow cytometry. Assays were performed on a Beckman Coulter Epics XL benchtop flow cytometer (Beckman Coulter, Brea, California, USA) and data were analyzed by FCS express software (De Novo Software, Toronto, Ontario, Canada). A minimum of 50 000 events was collected for each sample.

Antibodies and reagents

Anti-mCD11b-phycoerythrin (PE)-cyanin 5 (Cy5), anti-mF4/80-PE, anti-mGr1-PE, anti-mLy6C-fluorescein isothiocyanate (FITC), anti-mCD11c-PE, anti-mCD86-FITC, anti-mCD80-PE, anti-mI-antibody-PE, anti-mCD115-PE, anti-mCD124-PE, anti-mCD274-PE, anti-mCD31-PE, anti-mCD40-PE, anti-m tumour necrosis factor (TNF)-α-PE, anti-Arg-1-PE-Cy7, anti-m interferon (IFN)-γ-PE were purchased from BD Biosciences Pharmingen (San Diego, California, USA). Anti-CD4-FITC, anti-mCD4-PE, anti-mCD8-PE-Cy5 and anti-mLy6G-PE were purchased from eBioscience (San Diego, California, USA). Recombinant mouse M-CSF, granulocyte macrophage (GM)-CSF and IFN-γ were purchased from PeproTech (Rocky Hill, New Jersey, USA). Arg inhibitor nor-NOHA [half-maximal inhibitory concentration (IC_{50}) 10 μM] were purchased from Gene Operation (San Diego, California, USA).

Quantitative PCR (qPCR)

Total RNA of the sorted cells was extracted by Trizol (Invitrogen, Carlsbad, California, USA) and reverse transcription was performed with M-MLV superscript reverse transcriptase according to the manufacturer's instructions. Real-time PCR was carried out using multiple kits (SYBR Premix ExTaqTM, DRR041A; Takara Bio, Shiga, Japan) on CFX96 (Bio-Rad, Hercules, California, USA). The primers used in the present study are summarized in Table 1. The mRNA expression levels of each gene were normalized to the expression level of the housekeeping gene [hypoxanthine phosphoribosyltransferase 1 (HPRT) gene] and relative gene expression was calculated using the comparative Ct method.

TABLE 1 Primers used for qRT–PCR analysis

Genes	Primer sequence (5′–3′)
HPRT	
Forward primer:	AGT ACA GCC CCA AAA TGG TTAAG
Reverse primer:	CTT AGG CTT TGT ATT TGG CTT TTC
Arginase 1	
Forward primer:	CCA GAA GAA TGG AAG AGT CAG TGT
Reverse primer:	GCA GAT ATG CAG GGA GTC ACC
iNOS	
Forward primer:	CAC CAA GCT GAA CTT GAG CG
Reverse primer:	CGT GGC TTT GGG CTC CTC
COX2	
Forward primer:	CCT TCT CCA ACC TCT CCT ACT
Reverse primer:	ACC TTT TCC AGC ACT TCT TTTG
IDO1	
Forward primer:	CAA TCA AAG CAA TCC CCA CTG
Reverse primer:	AAA ACG TGT CTG GGT CCA C
NOX2	
Forward primer:	GAC CCA GAT GCA GGA AAG GAA
Reverse primer:	TCA TGG TGC ACA GCA AAG TGAT
TGF-β	
Forward primer:	GGC GGT GCT CGC TTT GTA
Reverse primer:	TCC CGA ATG TCT GAC GTA TTGA
IL-10	
Forward primer:	GGA GCA GGT GAA GAG TGA TT
Reverse primer:	CCC AAG GAG TTG TTT CCG TTA
IL-4	
Forward primer:	TTG TCA TCC TGC TCT TCT TTCTC
Reverse primer:	CAG GAA GTC TTT CAG TGA TGTGG
PD-L1	
Forward primer:	AGT CTC CTC GCC TGC AGA TAG
Reverse primer:	ACT CCA CCA CGT ACA AGT CC

Abbreviations: HPRT = hypoxanthine phosphoribosyl transferase; TGF-β = transforming growth factor beta; IL = interleukin; iNOS = inducible nitric oxide synthase; IDO1 = indoleamine-2,3-dioxygenase-1; COX2 = cyclooxygenase 2; NOX2 = NADPH oxidase 2; PD-L1 = programmed cell death ligand-1; qRT–PCR = quantitative polymerase chain reaction Genes Primer sequence (5′–3′).

Skin transplantation

Male B6 tail skin was grafted on the dorsal part of the female B6 recipients; 5×10^6 cells induced M-MDSCs per mouse were transferred to recipient mice by intravenous injection the day before transplantation. Skin graft survival was monitored by daily observations. Graft rejection was determined when less than 10% of the graft remained viable.

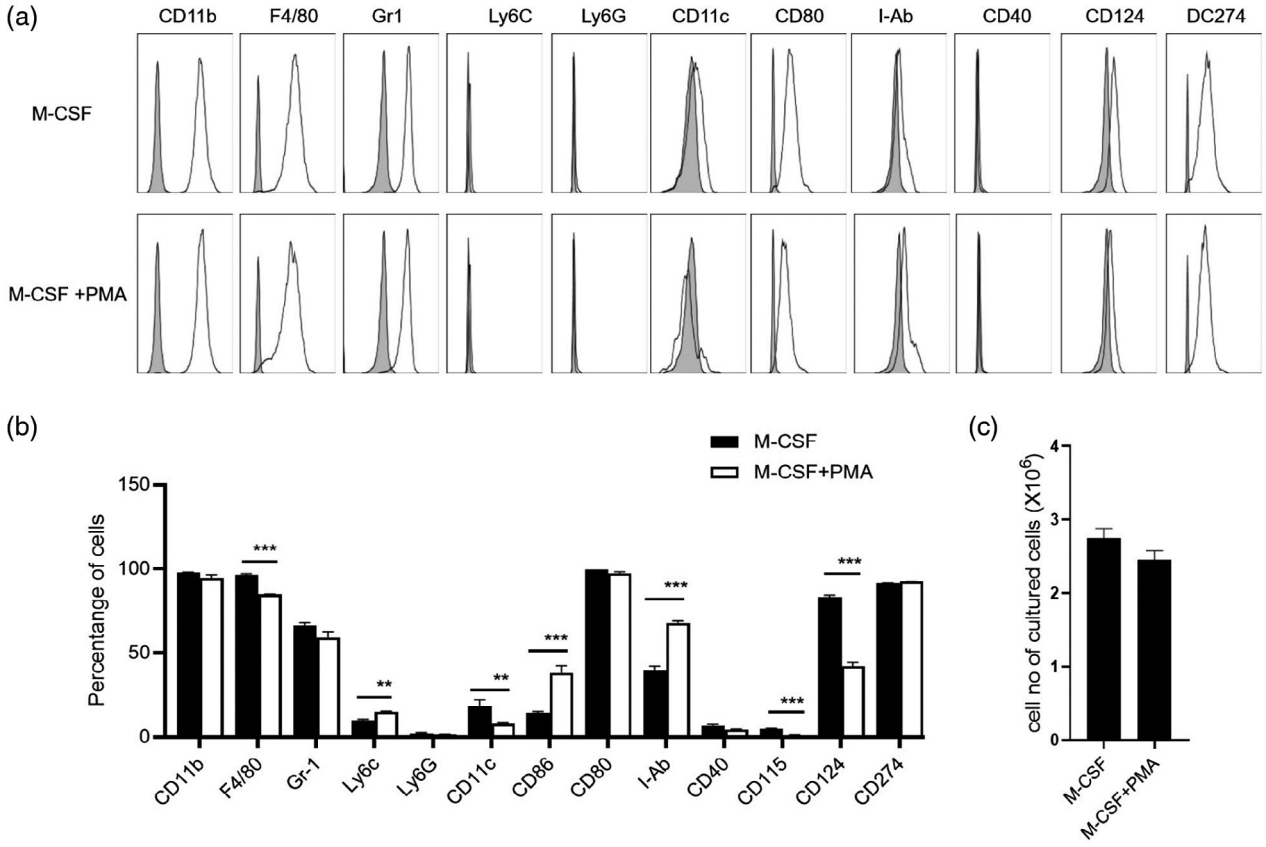

FIGURE 1 Phenotypical expression of myeloid-derived suppressor cells (MDSCs) induced by macrophage colony-stimulating factor (M-CSF) and M-CSF + phorbol 12-myristate 13-acetate (PMA). (a) Bone marrow cells were induced with M-CSF or M-CSF + PMA for 7 days, followed by staining with phycoerythrin (PE), fluorescein isothiocyanate (FITC) or PE-cyanin 5 (Cy5)-labeled anti-F4/80, CD11b, CD11c, Gr-1, lymphocyte antigen 6 (Ly6)C, Ly6G, I-Ab, CD80, CD86, CD40, CD115, CD124, CD274 or CD31 monoclonal antibody (mAb). The data were analyzed by flow cytometry. (b) Summary of the expression data for each phenotype. (c) The numbers of MDSCs induced by M-CSF and M-CSF + PMA, respectively, did not differ significantly

Statistical analysis

All data are presented as the mean ± standard deviation (SD). One- or two-way analyses of variance (ANOVA) analysis were used for comparison among multiple groups with SPSS version 17.0 software according to the type of data. Student's unpaired *t*-test for comparison of means was used to compare between two groups; *p*-values for comparison between graft survivals were determined using log-rank tests. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

PMA induces differentiation of monocytes into MDSCs

To investigate the effect of PMA on the differentiation of bone marrow mononuclear cells with M-CSF as growth factor, we used flow cytometry to determine the differentiation phenotypes of bone marrow cells induced by M-CSF alone and M-CSF combined with PMA, investigating the expression of the cell surface molecules F4/80, Gr-1, Ly6C, Ly6G, CD11c, CD86, CD80, CD40, I-Ab, CD115, CD124 (IL-4Rα), CD274 [programmed cell death ligand 1 (PD-L1)] and CD31. As shown in Figure 1, addition of PMA reduced the expression of CD115 and macrophage marker F4/80 ($p < 0.001$, Figure 1a,b). The expression of MDSC markers Ly6C was up-regulated ($p < 0.01$, Figure 1a,b), indicating that the induced cells were more inclined to the M-MDSC phenotype. The expression levels of co-stimulatory molecules CD86 were up-regulated ($p < 0.001$, Figure 1a,b), but CD40 and CD80 had no significant change. The expression of the IL-4 receptor CD124 related to T cell proliferation and activation was significantly decreased ($p < 0.001$, Figure 1a,b), while the expression of PD-L1 related to MDSC inhibitory function had no significant change. The expression of CD11c ($p < 0.01$, Figure 1a,b) and I-antibodies

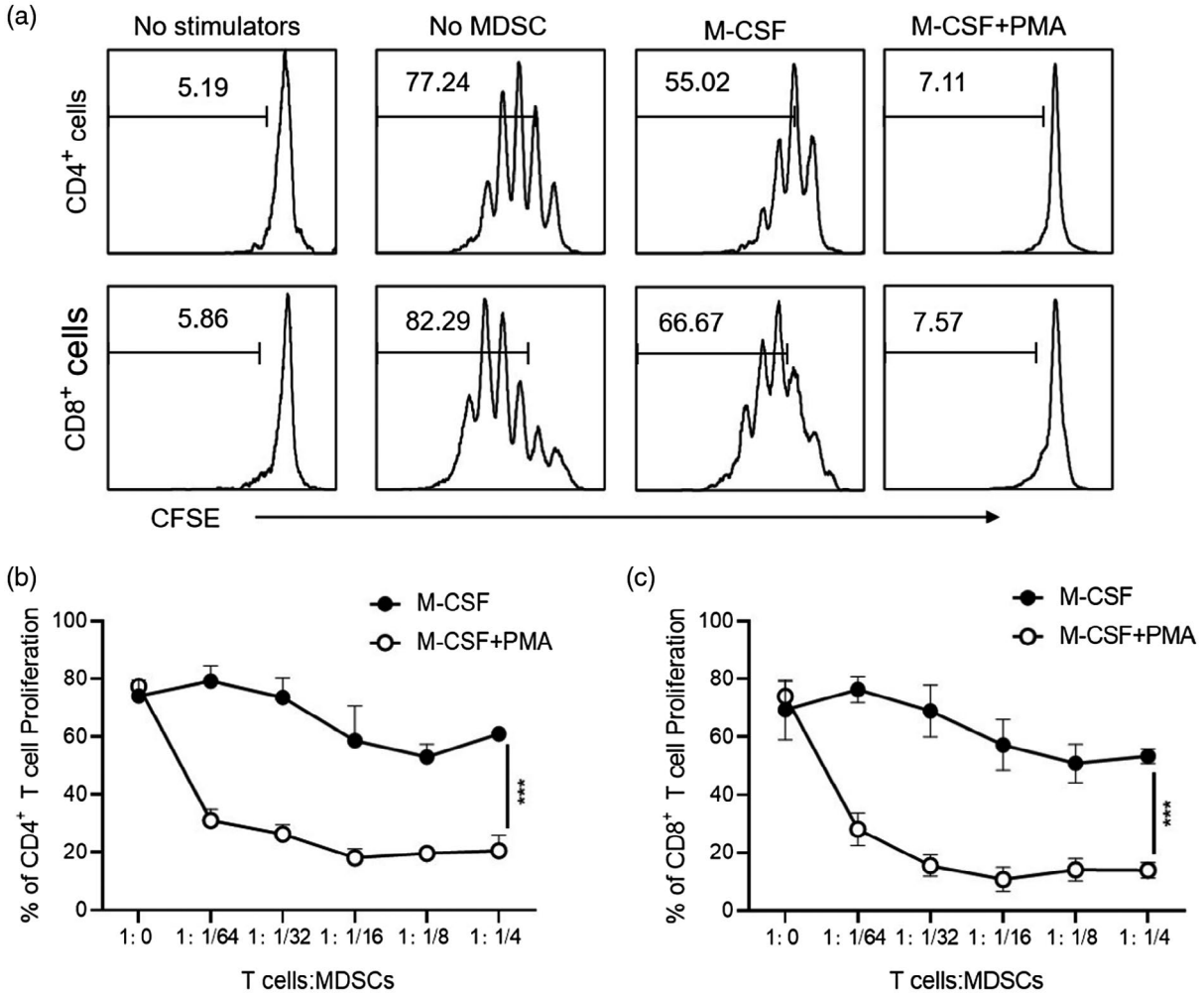

FIGURE 2 Myeloid-derived suppressor cells (MDSCs) induced by macrophage colony-stimulating factor (M-CSF) + phorbol 12-myristate 13-acetate (PMA) inhibited T cell proliferation stimulated by concanavalin A (ConA). MDSCs induced by M-CSF and M-CSF + PMA were co-cultured with different numbers of T cells for 3 days. T cell proliferation was detected by flow cytometry. (a) T cell: flow cytometry analysis of typical T cell proliferation at a ratio of T cells: MDSC of 1:1/8. The proliferation of $CD4^+$ (b) and $CD8^+$ (c) T cells stained with carboxyfluorescein succinimidyl ester (CFSE) in different ratios

($p < 0.001$, Figure 1a,b) related to antigen presentation were up-regulated. There were no significant differences in the number of cells induced by M-CSF and M-CSF + PMA (Figure 1c).

M-CSF + PMA-induces MDSCs suppress T cell functioning *in vitro*

To verify the immunosuppressive function of MDSCs induced by M-CSF + PMA, we co-cultured mouse lymphocytes and MDSCs *in vitro*, applying ConA as a stimulant to stimulate lymphocyte proliferation to show T cell proliferation. The cells induced by M-CSF were the control group and the MDSCs induced by M-CSF + PMA co-induction were the experimental group. Different ratios of MDSCs collected from the two groups were added to lymphocytes. The results showed that MDSCs induced by M-CSF + PMA could significantly inhibit CFSE-labeled $CD4^+$ or $CD8^+$ T cells, and the inhibitory effect was enhanced by increasing the number of MDSCs ($p < 0.001$, Figure 2). MDSCs could also significantly reduce the production of IFN-γ and TNF-α generated by the $CD4^+$ or $CD8^+$ T cells ($p < 0.05$, Figure 3a–f). Meanwhile, the expression of CD25 in T cells was significantly decreased after adding MDSCs ($p < 0.001$, Figure 3g–i), indicating that MDSCs could also inhibit T cell activation. MDSCs induced by M-CSF + PMA could promote the transformation of T cells into T_{reg} cells. The results showed that, compared with the control group, the number of $CD4^+FoxP3^+$ T_{reg} cells increased significantly in the experimental group ($p < 0.001$, Figure 3j,k). Taken together, MDSCs induced

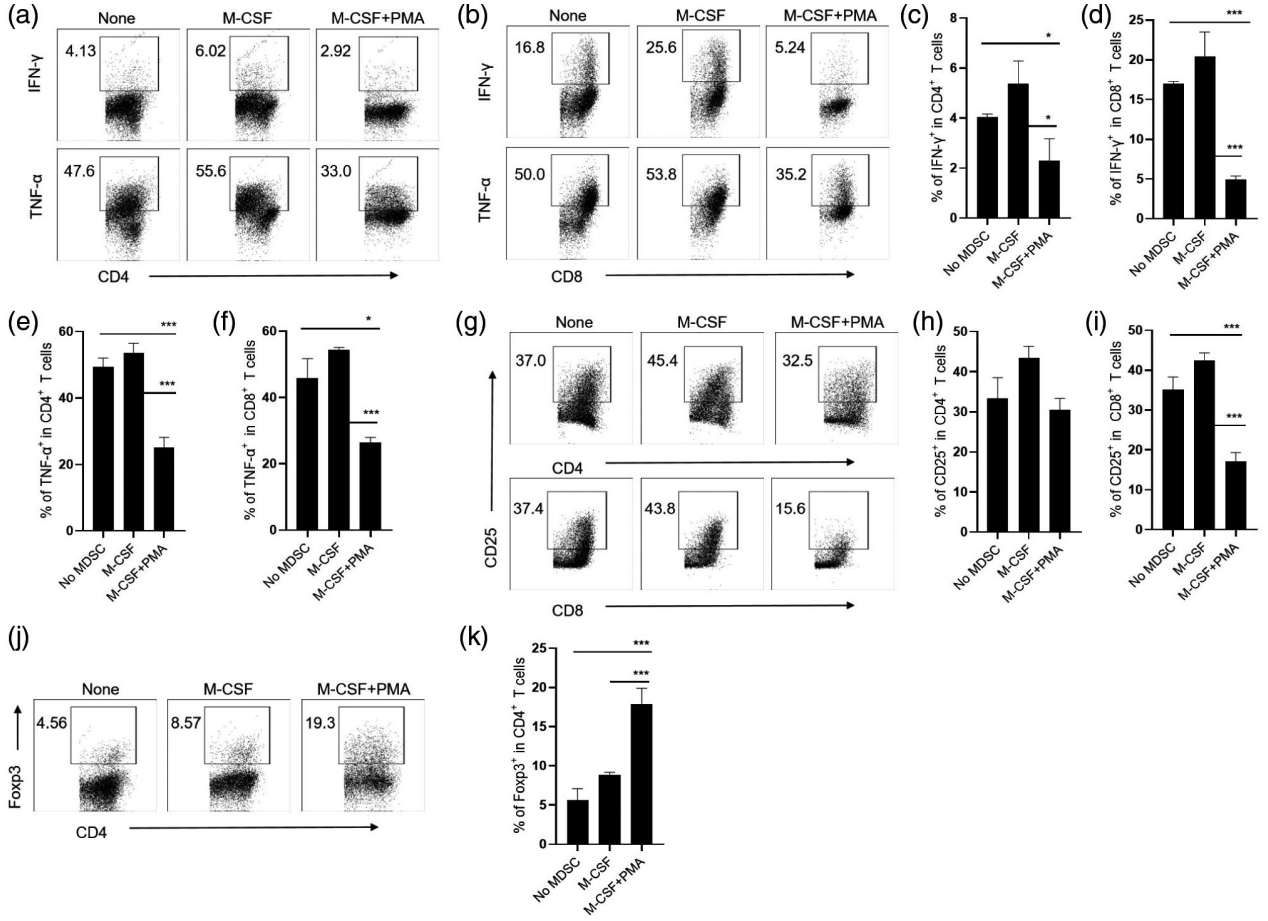

FIGURE 3 Myeloid-derived suppressor cells (MDSCs) induced by macrophage colony-stimulating factor (M-CSF) + phorbol 12-myristate 13-acetate (PMA) inhibited T cell proliferation stimulated by concanavalin A (ConA)-stimulated T cell proliferation, cytokine secretion and T cell transformation to regulatory T cells (T_{reg}). MDSCs induced by M-CSF + PMA were co-cultured with ConA-stimulated T cells for 3 days, then treated with PMA and ionomycin for 5 h. (a,b) Typical flow cytometry results showing cytokines secreted by T cells. The results show cytokine interferon (IFN)-γ (c,d) and tumor necrosis factor (TNF)-α (e,f) secreted by $CD4^+$ and $CD8^+$ T cells, respectively, stimulated by ConA. (g) CD25 expression in T cells co-cultured with MDSCs detected by flow cytometry. Statistical analysis of CD25 expression on $CD4^+$ (h) and $CD8^+$ (i) T cells. (j,k) Results and analysis of co-cultured MDSCs and T cells expressing $CD4^+$ forkhead box protein 3 $(FoxP3)^+$

by M-CSF + PMA could inhibit the proliferation and activation of T cells, reduce cytokine secretion and promote the T cells' transformation to T_{regs}, thus exerting an immunosuppressive function.

M-CSF + IFN-γ-induced MDSCs-mediated immunosuppression through Arg-1

To explore the immunoregulatory mechanism of M-CSF and PMA-induced MDSCs, we investigated the expression of immunoregulation-related mRNA by real-time PCR. The results showed that the expression level of Arg-1 in MDSCs induced by M-CSF + PMA was significantly higher than that in the control group induced by M-CSF alone ($p < 0.001$, Figure 4a). The expression levels of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2) and PD-L1 were slightly increased. There was no significant difference in the expression of COX2, HO-1, TGF-β, IL-10 and IL-4 between the two groups (Figure 4a). To verify whether the Arg-1 pathway played an essential role in the immunoregulatory activity of M cells induced by M-CSF + PMA, we added the Arg inhibitor NOR into the M-CSF + PMA induction system. We found that NOR significantly reversed the inhibition of T cell proliferation in a dose-dependent manner (Figure 4b–d). The results showed that the protein expression of Arg-1 in MDSCs induced by M-CSF + PMA was significantly higher than that in the group induced by M-CSF alone and the group induced by M-CSF + PMA + nor-NOHA ($p < 0.001$, Figure 4e,f),confirming that Arg-1 played

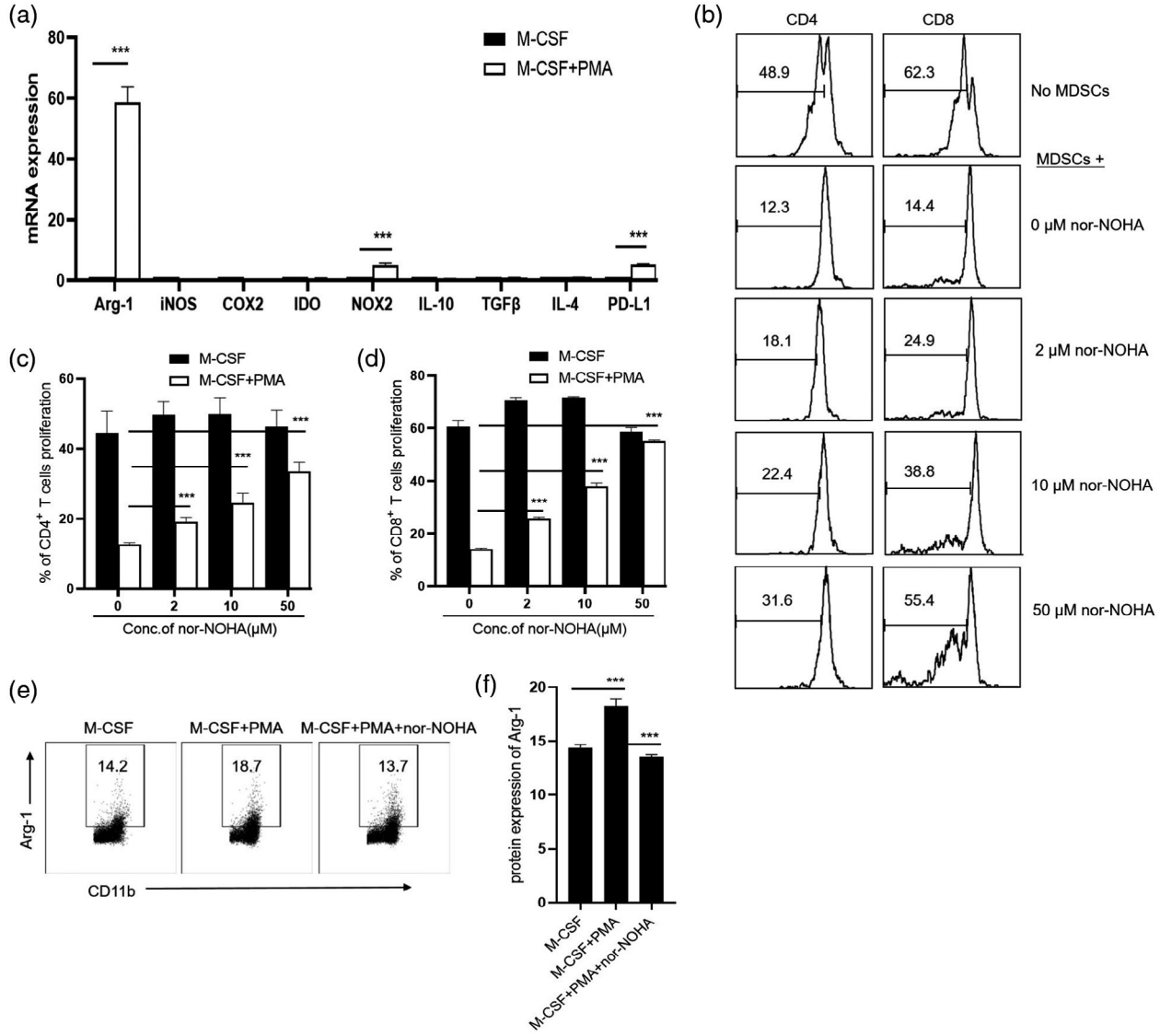

FIGURE 4 Myeloid-derived suppressor cells (MDSCs) induced by macrophage colony-stimulating factor (M-CSF) + phorbol 12-myristate 13-acetate (PMA) promoted T cell inhibitor through the arginase-1 (Arg-1) pathway. Real-time polymerase chain reaction (PCR) was used to detect the mRNA expression of Arg-1, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX2), indoleamine 2,3-dioxygenase 1 (IDO), NADPH oxidase (NOX2), interleukin (IL)-10, transforming growth factor (TGF)-β, IL-4 and programmed cell death ligand 1 (PD-L1) in MDSCs induced by M-CSF and M-CSF + PMA. The statistical results are shown in (a). Nor-NOHA, an inhibitor of Arg-1, reversed the inhibitory effect of MDSCs on $CD4^+$ (b) and $CD8^+$ (c) T cell proliferation (ratio of T cells: MDSC = 1:1/8). (d) Flow cytometry of co-culture of T cells with M-CSF + PMA-induced MDSCs after NOR treatment (ratio of T cells: MDSC = 1:1/8). (e,f) Results and analysis of protein expression of Arg-1

an important role in the immunoregulatory function of MDSCs induced by M-CSF + PMA.

MDSCs induced by M-CSF + PMA could prolong the survival time of skin grafts in mice

To explore whether MDSCs induced by M-CSF + PMA could stably regulate immune function *in vivo*, we adoptively transferred M-CSF + PMA-induced MDSCs or control differentiated myeloid cells to female recipient B6 mice via the tail vein, with each mouse receiving 5×10^6 cells. Twenty-four hours before the injection, the female mice received tail skin grafts from male B6 mice. The results showed that, compared with the control group, M cells induced by M-CSF + PMA could significantly prolong the survival time of skin grafts in mice ($p < 0.001$, Figure 5a,b). This was consistent with the previous results *in vitro*, indicating that MDSCs induced by M-CSF + PMA could stably exert an immunosuppressive function *in vivo* and *in vitro*.

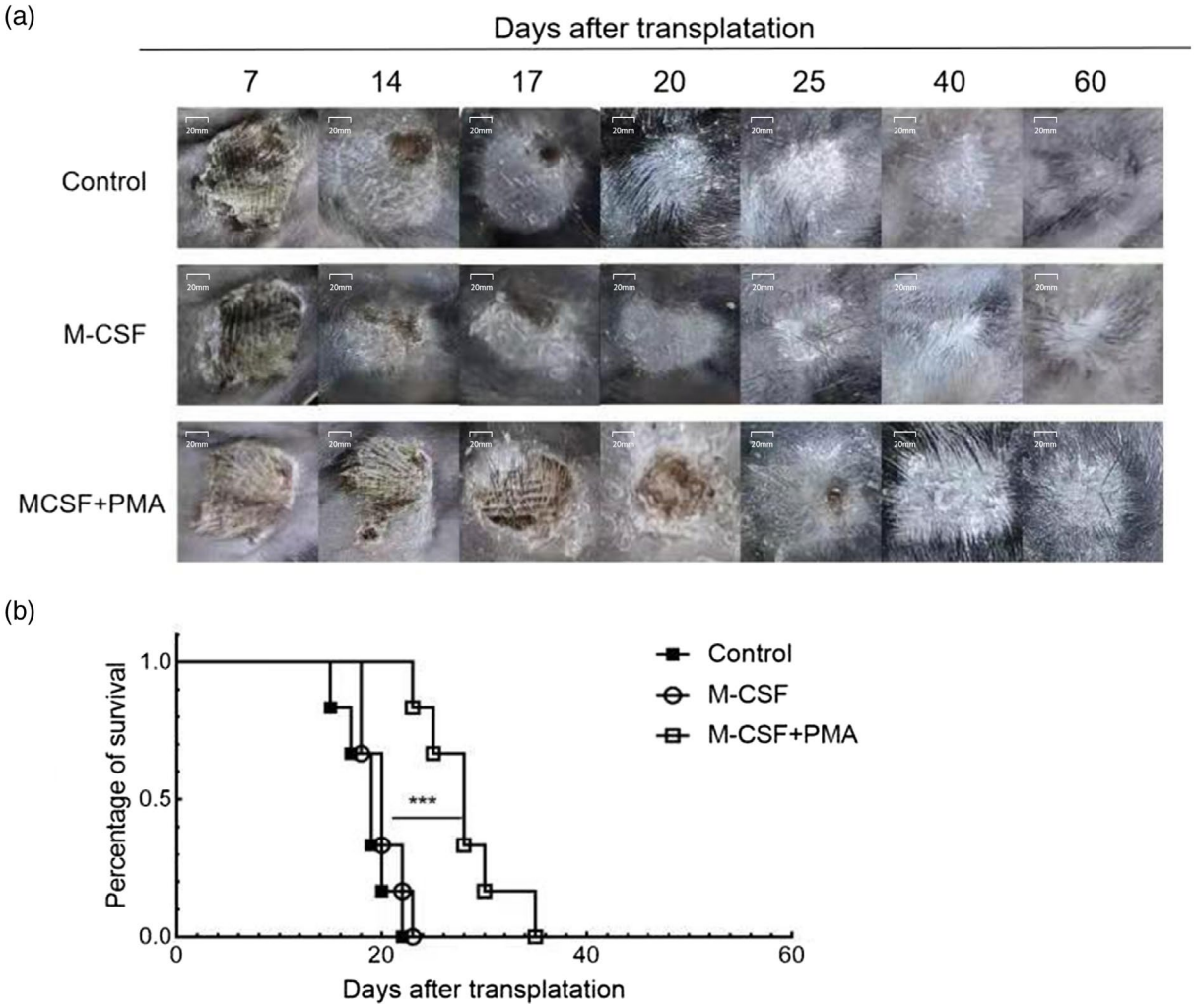

FIGURE 5 Myeloid-derived suppressor cells (MDSCs) prolong the survival time of skin allografts. Recipient female B6 mice received skin allografts from the tails of male mice. MDSCs from bone marrow or MDSCs induced by M-CSF + PMA were injected via the tail vein 24 h after the operation, with 5×10^6 cells in each mouse. Mice in the control group received the same amount of normal saline through the tail vein. (a) Photos of the tail skin graft were recorded. (b) The percentage of skin graft survival was recorded ($n = 6$)

DISCUSSION

MDSCs are immunomodulatory cells that negatively regulate the immune response, playing an essential role in maintaining immune tolerance and promoting graft survival [17]. Animal experiments have shown that *in vitro*-induced MDSCs can promote graft survival and prevent graft-versus-host disease. In addition to T_{regs}, DC_{regs} and M_{regs}, MDSC could potentially be applied in clinical treatment to inhibit rejection and even induce operative immune tolerance.

It has been reported that IL-6-induced MDSC could inhibit T cell function *in vitro* and prolong the survival of islet grafts *in vivo* [20]. In addition, in H–Y antigen-mediated skin graft rejection, it was found that MDSC could prolong graft survival [21]. We stimulate MDSC daily induced using the traditional 4-day method but we found it was not stable, suggesting that the *in-vivo* modulation was limited in skin transplantation models. Thus, we expanded the length of cytokine stimulation in culture from 1 to 4 days with a total culture time of 7 days, which was sufficient for myeloid cell differentiation [22]. In several studies, 4 days' culture with GM-CSF was used to induce MDSCs. Although the induced MDSCs showed a significant immunosuppressive effect *in vitro*, the effect was not significant *in vivo*.

M-CSF is a vital cytokine that can induce precursor cells to develop into monocytes and macrophages [23]. It has also been reported that M-CSF can induce MDSC with immunoregulatory activities *in vitro*. Macrophages induced by M-CSF have been shown to inhibit the proliferation of co-cultured T cells through the IDO pathway [24,25]. Therefore, we investigated the effects of a

different system, using myeloid colony-stimulating factor M-CSF combined with PMA to produce MDSCs with stable inhibitory activities.

In this study, it was found that M-MDSCs induced by M-CSF + PMA could inhibit T cell proliferation through Arg-1 and PMA treatment significantly enhanced the MDSC immunosuppressive activity. The cells expressed F4/80 and CD80 but not LY6G and CD11c, indicating that the cells were more macrophage-like M-MDSCs than the classical MDSCs found in tumors. We found that PMA could specifically increase the number of CD11b + Gr-$1^{int/low}$ MDSCs *in vitro* and significantly inhibit the proliferation of T cells. Investigation of the mechanism showed that the Arg-1 pathway participated in the immune regulatory activity of PMA-induced MDSCs as the production of Arg-1 in the PMA-induced bone marrow mesenchymal stem cell system increased significantly. Additionally, blocking the activity of Arg-1 by its inhibitor could block the PMA-induced MDSC immunosuppressive activity *in vitro*. Consistent with these *in-vitro* results, MDSCs induced by the adoptive transfer of PMA significantly prolonged the survival time of skin allografts *in vivo*.

CONCLUSIONS

A new method to induce MDSCs is reported in this study. The combination of M-CSF and PMA effectively induced cytokines inhibiting T cell proliferation and secretion and promoted the transformation of T cells into T_{regs}, following the immunoregulatory activities of M-MDSCs. The adoptive transfer of the induced cells significantly prolonged the survival time of allografts through the action of Arg-1. These results lay a foundation for the potential clinical application of M-CSF + PMA-induced M-MDSCs immunomodulation, providing a promising regulatory cell induction system for cell transplantation therapy.

ACKNOWLEDGEMENTS

This study was approved by the Ethics Committee of the Chao-Yang Hospital of Capital Medical University. Animal breeding, care and all experiments were performed in adherence to the guidelines of the Centre for Animal Experiment of Capital Medical University and approved by the Animal Ethics Committee (Institutional Review Board no.: AEEI-2021-108).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: H.W., Y.Z. and X.Z.; administrative support: Y.Z. and X.Z.; provision of study materials: H.W., J.W.J., Y.Z., X.Z.; collection and assembly of data: Haozhou Wang, JiaWei Ji, Yuan Zhuang; data analysis and interpretation: H.W., J.W.J., X.Z.; and manuscript writing: H.W.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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