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ORIGINAL ARTICLE

**Hiroshi Nakashima · Akira Tasaki · Makoto Kubo
Hideo Kuroki · Kotaro Matsumoto · Masao Tanaka
Masafumi Nakamura · Takashi Morisaki
Mitsuo Katano**

Effects of docetaxel on antigen presentation-related functions of human monocyte-derived dendritic cells

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Abstract *Purpose*: Docetaxel (TXT) is a unique chemotherapeutic agent that has been approved for treating various types of malignancies. TXT stabilizes microtubule assembly in cells and causes various dysfunctions of microtubule-dependent cellular events. Patients with advanced malignancies are beginning to receive TXT in combination with immunotherapy; however, the influence of TXT at clinically achievable serum concentrations (less than 10^{-6} *M*) on antigen presentation-related functions of human monocyte-derived dendritic cells (Mo-DCs) remains unclear. *Methods*: Immature Mo-DCs (imMo-DCs) were generated from peripheral blood monocytes with interleukin-4 and granulocyte-macrophage colony-stimulating factor in vitro. Mature Mo-DCs (mMo-DCs) were induced from imMo-DCs with tumor necrosis factor-α and prostaglandin E_2. *Results*: TXT at concentrations lower than 10^{-7} *M* did not significantly affect cellular viability, phagocytosis, or expression of antigen presentation-related molecules of Mo-DCs. In contrast, TXT at concentrations lower than 10^{-9} *M* significantly suppressed directional motility of imMo-DCs toward MIP-1α and of mMo-DCs toward MIP-3β. However, TXT had no effect on either CCR1 expression by imMo-DCs or CCR7 expression by mMo-DCs. No gross changes in the microtubule skeleton were evident by immunofluorescence microscopy after treatment with TXT at less than 10^{-8} *M*. However, reduced numbers of imMo-DCs with podosomes localized primarily in one cell region were observed. *Conclusions*: The present results indicate that different concentrations of TXT influence antigen presentation-related functions differently. In particular, TXT at relatively low therapeutic doses disrupts chemotactic motility of Mo-DCs.

Keywords Taxane · Non-directional migration · Directional migration · Microtubules · Immunotherapy

H. Nakashima · A. Tasaki · M. Kubo · H. Kuroki
K. Matsumoto · M. Nakamura · T. Morisaki
M. Katano (✉)
Department of Cancer Therapy and Research,
Graduate School of Medical Sciences,
Kyushu University, 3-1-1 Maidashi,
Higashiku, Fukuoka City 812-8582, Japan
E-mail: mkatano@tumor.med.kyushu-u.ac.jp
Tel.: +81-92-6426941
Fax: +81-92-6426221

M. Tanaka
Department of Surgery and Oncology,
Graduate School of Medical Sciences,
Kyushu University, 3-1-1 Maidashi,
Higashiku, Fukuoka City 812-8582, Japan

Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and are capable of inducing primary sensitization against specific antigens in naive T cells [3]. Immature DCs (imDCs) exist in most tissues. They capture and process antigens. Following activation, they display these antigens in the form of MHC-peptide complexes at their surface [9]. Mature DCs (mDCs) enter lymphatic vessels, migrate to T-dependent areas of secondary lymphoid organs, and stimulate naive T cells [2]. Thus, the ability of DCs to migrate is crucial to the transmission of immunological events in peripheral tissues to secondary lymphoid organs.

It is now possible to generate in vitro DC-like APCs (Mo-DCs) from human peripheral blood mononuclear cells (PBMCs) with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) [29]. Immature Mo-DCs (imMo-DCs) and mature Mo-DCs (mMo-DCs) also migrate toward MIP-1α and MIP-3β, respectively [8, 22]. Mature Mo-DCs are able to enter lymphatic vessels and migrate to regional lymph nodes in animal models, and mMo-DCs might be involved in induction of tumor-specific cytotoxic T lymphocytes (CTLs) [11, 44]. Based on these experimental findings, a number of studies have shown that

subcutaneous injection of mMo-DCs loaded with tumor-associated antigens leads to antitumor immune responses in patients with various types of malignancies [4, 11, 13, 28]. In these DC-based vaccine therapies, the ability of injected mMo-DCs to migrate plays an essential role in CTL induction.

For a cell to invade, the front of the cell must protrude and attach to a substrate and then the rear part of the cell must be able to retract. These processes are directly driven by the actin cytoskeleton [35, 36]. Podosomes are unique actin-rich adhesion structures of monocyte-derived cells [7]. They are highly dynamic and actively engage in matrix remodeling and tissue invasion [25]. Microtubules also play a role in the locomotion of most, but not all, cell types, and they may be involved in the coordination of the direction of cell movement [15, 33]. Linder et al. [26] have shown that microtubules are essential for podosome formation in primary human macrophages. These findings indicate the significance of actin and microtubule cytoskeletons in cell motility. Thus, compounds that damage actin or microtubules may affect cell motility.

Docetaxel (TXT) is a new chemotherapeutic agent that has been approved for treatment of various types of malignancies [16, 18–20, 23, 30]. TXT is a semisynthetic taxane derived from the needles of the European yew (*Taxus baccata*). It binds to tubulin, leading to microtubule stabilization, mitotic arrest, and subsequent cell death [14, 17, 39]. TXT has been reported to affect the migratory capacity of certain cells such as endothelial cells [21], smooth muscle cells [1], some cancer cells [5, 37, 40], and neutrophils [32]. However, there is limited information concerning the influence of TXT on Mo-DC motility.

Cancer patients receiving chemotherapeutic agents, including TXT, sometimes also receive DC vaccine therapy. In addition, recent animal experiments suggest that chemotherapeutic agents administered in combination with Mo-DC-based vaccine therapy may be effective for treating cancer patients with multiple drug resistance [41]. Thus DC vaccine therapy is likely to become a more common component in regimens for treatment of cancer. Because TXT affects microtubule function, which is important for motility of DCs used in vaccine therapy, we investigated the effect of TXT on immunological functions of Mo-DCs, with particular emphasis on Mo-DC motility.

Materials and methods

Reagents and antibodies

TXT was purchased from Rhone-Poulenc-Rorer (Antony, France). Streptococcal preparation OK-432 was provided by Chugai Pharmaceutical Company (Tokyo, Japan). Human MIP-1α and MIP-3β were purchased from Diaclone Research (Besançon, France). The following monoclonal antibodies (mAb), conjugated with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE), were purchased from BD Biosciences Pharmingen (San Diego, Calif.): CD14, CD80, CD83, CD86, HLA-DR. Unlabeled mouse anti-human CCR1 and CCR7, FITC-conjugated gout anti-mouse immunoglobulins, and isotype controls, IgG1 and IgG2a, were also purchased from BD Biosciences Pharmingen.

Mo-DC preparation

Mo-DCs were generated from the PBMCs of healthy volunteers as previously described with minor modifications [43]. Briefly, PBMCs were suspended in RPMI 1640 medium (Sanko Pure Chemicals, Tokyo, Japan) with 10% fetal calf serum (FCS) (referred to as RPMI-FCS medium) for 4 h at 37°C, and the adherent cells were cultured in RPMI medium supplemented with 200 ng/ml GM-CSF (GeneTech, Beijing, China) and 500 U/ml IL-4 (Osteogenetics, Würzburg, Germany). On day 7, non-adherent cells were collected and further purified by negative selection with magnetic beads coated with mouse monoclonal anti-CD2, anti-CD3, and anti-CD19 antibodies (Dynabeads, Dynal Biotech, Oslo, Norway). This depletion procedure yielded over 90% $CD14^-$, $CD80^+$, and $HLA\text{-}DR^+$ imMo-DCs as assessed by fluorescence-activated cell sorting (FACS) with a FACS Calibur (Becton Dickinson, Franklin Lakes, N.J.). To induce maturation, imMo-DCs were cultured with RPMI-FCS medium supplemented with tumor necrosis factor-α (TNF-α, 200 U/ml; Dainippon Pharmaceutical, Osaka, Japan) and prostaglandin E_2 (PGE_2, 1 μg/ml; Sigma, St. Louis, Mo.) for two additional days [27]. These cells were used as mMo-DCs [24].

Cell viability and detection of apoptosis

ImMo-DCs were seeded into 96-well plates and cocultured with the indicated concentrations of TXT at 37°C in RPMI 1640 supplemented with 1% human albumin (hereafter referred to as RPMI-Alb medium). Following incubation for 24 h, cell viability was determined by the 3-[4,5-dimethythiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) assay. Percent cell viability is expressed as the mean ± SD of three independent wells.

Morphological changes in the nuclear chromatin of cells undergoing apoptosis were detected by staining with the membrane-permeable dye Hoechst 33342 (Wako Chemicals, Osaka, Japan). Briefly, cells were plated in 96-well plates, treated with TXT for 24 h, stained with Hoechst 33342, and then observed by fluorescence microscopy. A total of 300 cells were counted in three randomly chosen fields at ×100 magnification. Cells with condensed or fragmented nuclei were considered to be apoptotic. The proportions of apoptotic cells are

expressed as mean ± SD percentages of three independent wells.

Immunofluorescence microscopy

ImMo-DCs and mMo-DCs were pretreated with the indicated concentrations of TXT at 37°C for 24 h. After incubation, cells were washed with RPMI to eliminate TXT. Washed cells were allowed to adhere to glass coverslips at 37°C overnight. Cells were then fixed with 100% methanol at −20°C for 5 min. After washing with PBS containing 0.1% Tween 20 (Nacalai Tesque, Kyoto, Japan), non-specific binding was blocked with 10% goat serum in PBS. Actin and microtubule cytoskeleton were visualized by immunofluorescence staining with mouse mAbs to actin (1:200; Sigma) and to α-tubulin (1:500; Sigma), respectively. Alexa 594-conjugated goat anti-mouse IgG antibody (Wako Chemicals) was used as a second antibody. Fluorescence signals were detected with a Radiance 2000 confocal laser-scanning microscope (Bio-Rad Laboratories, Hercules, Calif.). Images were processed with Laser Sharp 2000 software (Bio-Rad Laboratories).

Migration of Mo-DCs

Migration of Mo-DCs was determined by counting the number of cells that migrated through Transwell inserts with filter membranes of pore size 8 mm (BD Biosciences Pharmingen). The effect of TXT on non-directional Mo-DC migration was determined as follows. Mo-DCs treated with designated doses of TXT for 24 h were suspended at a concentration of 2×10^5 cells/ml in RPMI-Alb medium. Cell suspension (500 μl) was added to the upper compartment, and RPMI-Alb medium (400 μl) was added to the lower compartment. The cells were incubated at 37°C for 6 h. After incubation, the filter was fixed with 100% methanol and stained with Giemsa solution, and the cells on the upper surface were completely removed. Mo-DCs that had migrated from the upper side to the lower side of the filter were counted under a light microscope at a magnification of ×200. Non-directional migration is expressed as the mean ± SD migrating cell number of five microscopic fields.

The procedure for determining the effect of TXT on directional migration was the same as that for determining non-directional migration; however, to determine directional migration of imMo-DCs or mMo-DCs, MIP-1α (10 ng/ml) or MIP-3β (100 ng/ml), respectively, was added to the lower compartment.

Chemokine-induced invasiveness of Mo-DCs

Chemokine-induced invasiveness of Mo-DCs was measured by the invasion of cells through Matrigel-coated Transwell inserts [42]. Briefly, the upper surface of the filter (pore size 8.0 μm; BD Biosciences Pharmingen) was coated with basement membrane Matrigel (BD Biosciences Pharmingen) at a concentration of 250 μg/cm^2 and air-dried overnight at room temperature. The invasion assay was similar to the directional migration assay described above. MIP-1α (for imMo-DCs) or MIP-3β (for mMo-DCs) was added to the lower compartment. After 24 h, Mo-DCs that had migrated from the upper side to the lower side of the filter were counted under a light microscope at a magnification of ×200. Chemokine-induced invasion is expressed as the mean ± SD migrating cell number of five microscopic fields.

Evaluation of phagocytosis

Immature Mo-DCs (1×10^5/well) pretreated with designated doses of TXT for 24 h were suspended in RPMI 1640 medium with FITC-conjugated dextran (FITC-DX, Sigma) and incubated for 12 h. The percentage of imMo-DCs that captured FITC-DX was determined by examining 100 imMo-DCs under a fluorescence microscope. The percent phagocytosis is expressed as the mean ± SD of three wells.

Alternatively, imMo-DCs were labeled with PE-conjugated anti-HLA-DR mAb. The fluorescence-labeled imMo-DCs were cultured with FITC-DX for 12 h at 37°C or 4°C, washed, and applied to a FACS Calibur flow cytometer. The fluorescence intensity was analyzed with CellQuest (Becton-Dickinson). FITC-positive cells in gated HLA-DR-positive imMo-DC populations were defined as dextran-captured imMo-DCs.

Expression of antigen presentation-related antigens of Mo-DCs

For analysis of the effect of TXT on expression of maturation-related molecules of Mo-DCs, the following mouse anti-human mAbs, conjugated with either FITC or PE, were used: CD14, CD80, CD83, CD86, and HLA-DR. Unlabeled mouse anti-human mAbs CCR1 and CCR7 were visualized with FITC-conjugated anti-mouse immunoglobulins. Isotype controls, IgG1, and IgG2a were also included. Cells were stained at a concentration of 1×10^5 in 100 μl. Samples were incubated with the conjugated mAbs for 60 min at 4°C and then washed twice with PBS containing 3% bovine serum albumin (BSA; Sigma) and 0.1% NaN_3 (Sigma). The samples were analyzed with a FACS Calibur flow cytometer and CellQuest.

IL-12 secretion in Mo-DCs

Because our previous study had shown that OK-432 induces IL-12 secretion in imMo-DCs [24, 31], imMo-DCs

(1×10^5/ml) were incubated with the indicated doses of TXT for 24 h at 37°C, washed to eliminate TXT, and then incubated with OK-432 (0.02 KE/ml) for 24 h. Cell-free supernatants were collected by centrifugation and stored at −80°C. The concentration of IL-12 p40 and p70 in the supernatants was determined using an enzyme-linked immunosorbent assay (ELISA) kit specific for measuring IL-12 p40 and p70 (BioSource International, Camarillo, Calif.) according to the manufacturer's instructions. The detection limit for IL-12 p40 and p70 was 7.8 pg/ml and 1.56 pg/ml, respectively. Concentrations of IL-12 p40 are expressed as the mean ± SD of the data from three independent experiments.

Mixed lymphocyte reaction

Mixed lymphocyte reaction (MLR) was carried out in U-bottomed 96-well plates with a total volume of 200 µl per well. Irradiated imMo-DCs (2×10^4 cells), which were treated with designated doses of TXT for 24 h at 37°C, were suspended in RPMI-Alb medium. Allogenic PBMCs (1×10^5) were used as responders. Wells were pulsed 3 days after the initial culture with 1 µCi (0.037 MBq) of [^{3}H]-thymidine (Amersham Pharmacia Biotech, Piscataway, N.J.). [^{3}H]-Thymidine incorporation was measured 24 h after the addition of [^{3}H]-thymidine with a liquid-scintillation counter (Beckman Coulter, Palo Alto, Calif.). [^{3}H]-Thymidine uptake is expressed as the mean ± SD counts per minute of three wells.

Statistical analysis

Statistical analysis was performed with the unpaired two-tailed Student's t test; $P < 0.05$ was considered significant.

Results

Effects of TXT on the viability of Mo-DCs

Achievable serum concentrations of TXT in the clinical setting are lower than 10^{-6} M and a concentration of 10^{-9} M is maintained for about 72 h [6]. When imMo-DCs were exposed to TXT at concentrations greater than 10^{-6} M for 24 h, viability decreased (Fig. 1a). The pattern of cellular death was apoptosis (Fig. 1b). Data are representative of six independent experiments with imMo-DCs generated from three different healthy donors.

Effects of TXT on cytoskeletal organization of imMo-DCs

Untreated imMo-DCs were oval in shape, indicating a polarized morphology (Fig. 2a). After treatment with

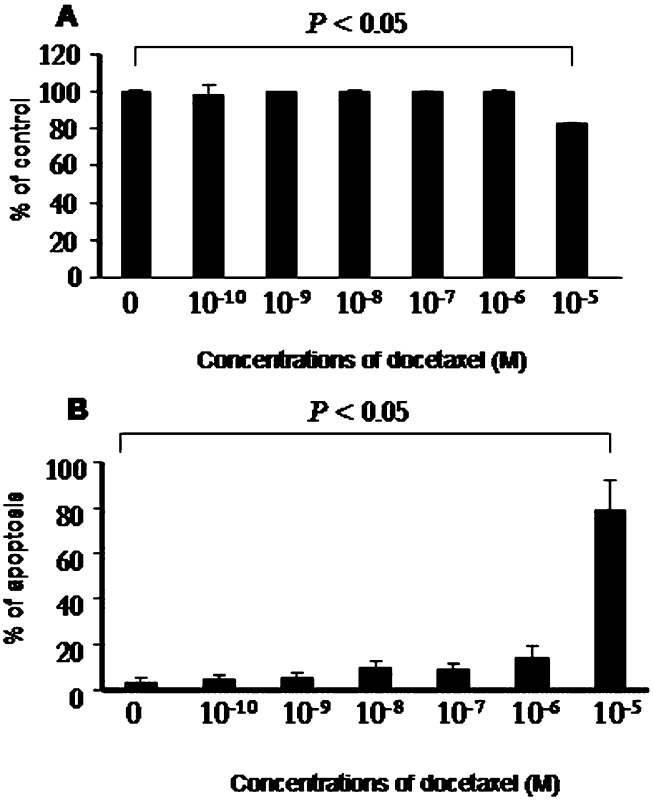

Fig. 1 Effect of TXT on viability of imMo-DCs. **a** The MTT assay was performed after 24 h of TXT treatment. Each experiment was performed in triplicate and the average optical density (OD) at 570 nm was calculated. The data presented are the means ± SD of three different wells. **b** Cells were stained with Hoechst 33342 and examined by fluorescence microscopy. A total of 300 cells were counted in three randomly chosen fields at ×100 magnification. Cells with condensed or fragmented nuclei were considered to be apoptotic. The data presented are percent apoptosis and are the means ± SD of three independent wells

TXT at concentrations greater than 10^{-8} M, imMo-DCs became rounded (Fig. 2d). The effect of TXT on cytoskeletal organization was examined by immunohistochemistry as described in "Materials and methods" (Fig. 2). In more than 90% of untreated imMo-DCs, multiple clear small actin foci resembling podosomes were found (Fig. 2b). Clusters of podosomes were localized primarily to one region in most untreated imMo-DCs, whereas in about 70% of imMo-DCs treated with TXT at 10^{-8} M, podosome clustering was mostly absent (Fig. 2e). In particular, almost all imMo-DCs treated with TXT at concentrations greater than 10^{-7} M failed to spread significantly on Matrigel-coated glass coverslips and lacked podosomes (Fig. 2h). No apparent change in microtubule cytoskeleton was found in cells treated with concentrations lower than 10^{-8} M TXT (Fig. 2f); however, an interwoven fabric of highly concentrated filaments or dense peripheral banding of filaments was found in almost all cells treated with greater than 10^{-7} M TXT (Fig. 2i).

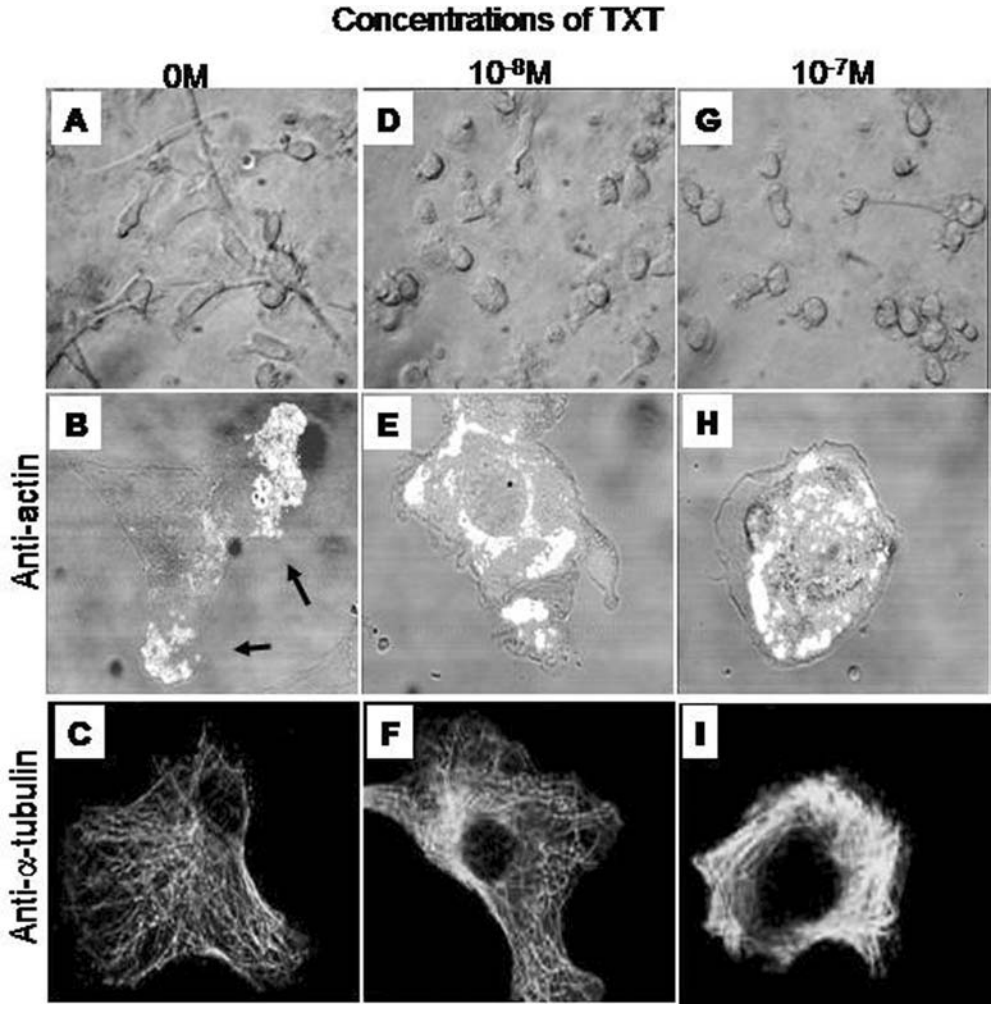

Fig. 2 Effect of TXT on cytoskeletal organization of imMo-DCs. ImMo-DCs were treated with the indicated doses of TXT for 24 h and observed under a phase contrast microscope (**a**, **d**, **g**; ×400) and confocal laser microscope (**b**, **c**, **e**, **f**, **h**, **i**; ×2000). Specimens were stained with either anti-actin mAb (**b**, **e**, **h**) or anti-α-tubulin mAb (**c**, **f**, **i**). *Arrows* indicate podosomes

Effects of TXT on the motility of Mo-DCs

A migration assay was performed with a modified Boyden chamber technique as described in "Materials and methods". TXT at 10^{-8} *M* significantly decreased non-directional motility of both imMo-DCs and mMo-DCs (Fig. 3a,b). TXT at 10^{-10} *M* significantly decreased MIP-1α-induced directional motility of imMo-DCs (Fig. 3c), and TXT at 10^{-9} *M* significantly decreased MIP-3β-induced directional motility of mMo-DCs (Fig. 3d). The data presented are representative of three independent experiments with Mo-DCs generated from three different healthy donors.

Because CCR1 and CCR7 are receptors for MIP-1α and MIP-3β, respectively [8], we examined by FACS analysis the effect of TXT on expression of CCR1 on imMo-DCs and CCR7 on mMo-DCs. TXT at concentrations lower than 10^{-7} *M* did not significantly affect the expression CCR1 and CCR7 on Mo-DCs (data not shown).

Effect of TXT on chemokine-induced invasiveness of Mo-DCs

To invade a new territory, Mo-DCs must be able to penetrate the matrix, especially in response to chemokines. In this study, chemokine-induced invasive ability (chemo-invasive ability) was determined by a Matrigel invasion assay as described in "Materials and methods". TXT at 10^{-9} *M* significantly decreased the chemoinvasive ability of imMo-DCs or mMo-DCs toward MIP-1α or MIP-3β, respectively (Fig. 4a,b). The data presented are representative of three independent experiments with Mo-DCs generated from three different healthy donors.

Effect of TXT on phagocytic activity of Mo-DCs

Phagocytic ability of imMo-DCs was determined with FITC-DX by both fluorescence microscopy (data not

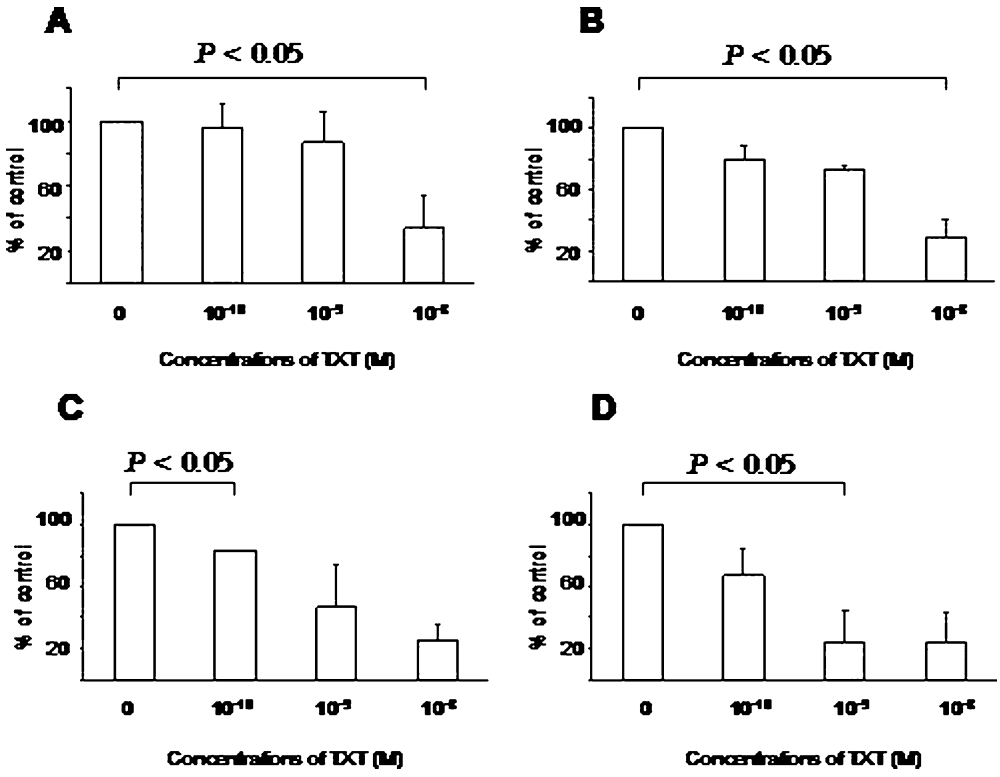

Fig. 3 Effect of TXT on the motility of Mo-DCs. The data presented are the ratios of TXT-treated migrating Mo-DCs to untreated Mo-DCs. **a** Non-directional migration of imMo-DCs; **b** non-directional migration of mMo-DCs; **c** directional migration of imMo-DCs against MIP-1α; **d** directional migration of mMo-DCs against MIP-3β

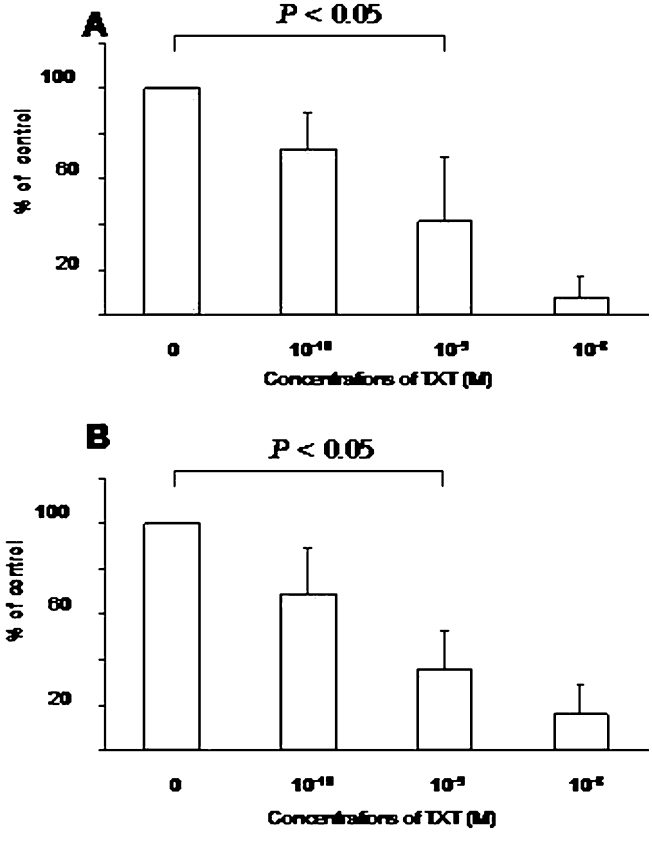

Fig. 4 Effect of TXT on chemoinvasive ability of Mo-DCs as determined by a Matrigel invasion assay. The data presented are the ratios of TXT-treated migrating Mo-DCs to untreated Mo-DCs. **a** Chemokine-induced invasion of imMo-DCs toward MIP-1α; **b** chemokine-induced invasion of mMo-DCs toward MIP-3β

shown) and FACS analysis (Fig. 5) as described in "Materials and methods". TXT at concentrations lower than 10^{-7} *M* did not affect phagocytic ability of imMo-DCs (Fig. 5). The data presented are representative of three independent experiments with Mo-DCs generated from three different healthy donors. When imMo-DCs were cultured with FITC-DX at 4°C (Fig. 5), their phagocytic ability was less than 5%, indicating capture of dextran by imMo-DCs rather than non-specific binding of dextran with imMo-DCs.

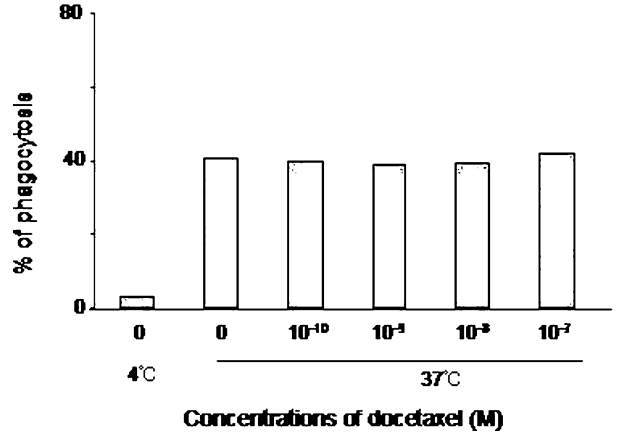

Fig. 5 Effect of TXT on phagocytosis by imMo-DCs. Cells were cocultured for 12 h with FITC-DX and subjected to FACS analysis. The data presented are representative of three different experiments

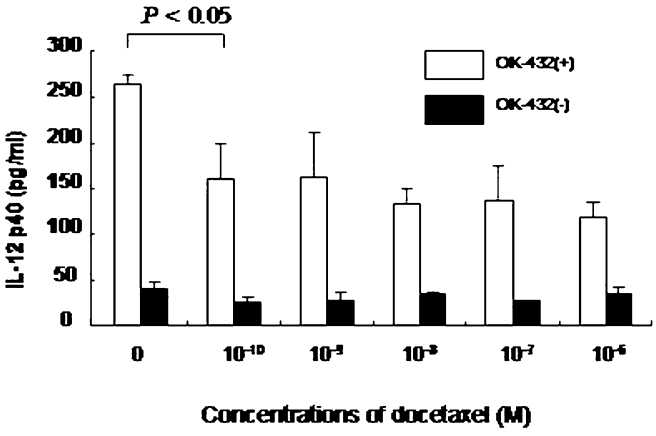

Fig. 6 Effect of TXT on IL-12 production by OK-432-stimulated Mo-DCs. ImMo-DCs treated with the indicated doses of TXT for 24 h were incubated with or without 0.02 KE/ml OK-432 at 37°C for 24 h. IL-12 p40 concentrations in the culture medium were determined by ELISA. The data presented are the means ± SD of three different wells

Effect of TXT on the expression of antigen presentation-related antigens of Mo-DC

TXT at 10^{-7} *M* did not affect the expression of antigen presentation-related antigens, including CD14, HLA-DR, CD80, and CD83, on either imMo-DCs or mMo-DCs (data not shown).

Effect of TXT on IL-12 production by Mo-DCs

When imMo-DCs capture antigens, such as streptococcal preparation OK-432, they secrete IL-12, which plays an important role in induction of CTLs [38]. Based on this finding, we examined the effect of TXT on IL-12 p40 production by imMo-DCs stimulated with OK-432. TXT at 10^{-10} *M* reduced IL-12 p40 production of imMo-DCs (Fig. 6) after coincubation with OK-432, but IL-12 p40 production from imMo-DCs without OK-432 stimulation was not affected by TXT. The data presented are representative of three independent experiments with Mo-DCs generated from three different healthy donors. OK-432 induced a considerable amount of IL-12 p70 in one of three cases. In this case, IL-12 p70 was also reduced at concentrations as low as 10^{-9} *M* (data not shown).

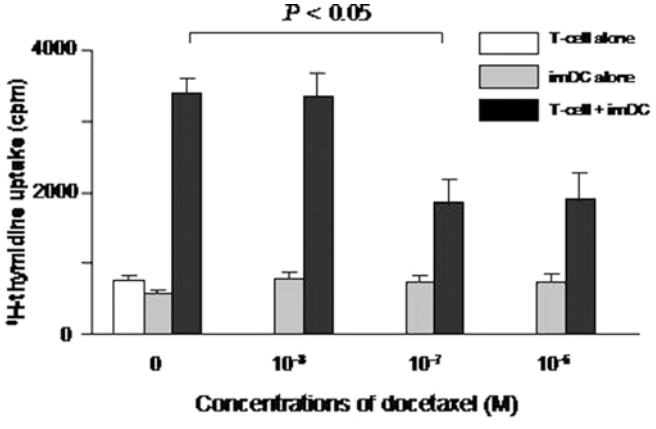

Fig. 7 Effect of TXT on allogenic T-cell stimulation by imMo-DCs. [^{3}H]-Thymidine incorporation into allogenic T cells cultured with TXT-treated imMo-DCs is shown. The data presented are the means ± SD of three different wells

Effect of TXT on imMo-DC-mediated allogenic T-cell response

To determine whether expression of antigen presentation-related molecules on imMo-DCs affects antigen-presenting potential, the ability of imMo-DCs to stimulate allogenic T-cell proliferation was determined by MLR as described in "Materials and methods". TXT at concentrations greater than 10^{-7} *M* significantly decreased [^{3}H]-thymidine uptake, suggesting that TXT decreases the ability of imMo-DCs to stimulate allogenic T-cell proliferation (Fig. 7). The data presented are representative of three independent experiments with allogenic PBMCs and Mo-DCs generated from three different healthy donors.

Discussion

In the present study, we showed that TXT at different concentrations produced different effects on antigen presentation-related functions of Mo-DCs. The effect of TXT on Mo-DC motility is particularly noteworthy.

ImDCs are capable of antigen capture [9]. However, DCs must mature and be adequately activated to become effective APCs. DCs that are insufficiently mature may induce immune tolerance rather than immune responsiveness [3]. It is generally accepted that antigen presentation-related functions of Mo-DCs are closely similar to those of DCs. Since TXT of 10^{-7} *M* did not affect Mo-DC viability, phagocytosis, or expression of antigen presentation-related surface molecules (Figs. 1 and 5), it is unlikely that patients receiving TXT in the clinic would have impaired imDC or imMo-DC function. Nevertheless, we have to recognize the possibility that TXT at high doses may weaken antigen-presenting ability, because TXT at 10^{-7} *M* suppressed allogenic T-cell proliferation by imMo-DCs (Fig. 7).

We wish to emphasize that TXT even at concentrations lower than 10^{-8} *M* impaired the motility of both imMo-DCs and mMo-DCs (Fig. 3). ImDCs respond to a large spectrum of chemokines through specific receptors. MIP-3α appears to be the most powerful chemokine guiding imDCs [8]. However, MIP-3α has no effect on imMo-DCs [3, 8]. In this

study, therefore, we chose MIP-1α as the chemokine for imMo-DCs [3]. Hotchkiss et al. [21] have reported that TXT at a very low concentration (10^{-12} *M*) reduces the migratory ability of endothelial cells. Substoichiometric binding of taxanes suppresses microtubule dynamics [10]. These findings imply that drug-mediated effects on microtubule plasticity/dynamics rather than on gross microtubule organization or expression are sufficient for inhibition of cell locomotion. Cell migration usually requires adhesion of cells to matrices such as integrins and fibronectin.

Podosomes are actin-rich adhesion structures found in monocyte-derived cells [12]. Although the roles of podosomes in cell spreading and migration and the mechanisms of their formation or dissolution are not yet clearly understood, a close relationship between microtubules and podosomes has been suggested [34]. For example, partial disassembly of microtubules leads to a more random pattern of podosome distribution [26]. Recently, it has been shown that microtubules are essential for podosome formation in human macrophages: freshly isolated monocytes undergoing adhesion fail to develop podosomes when treated with microtubule-depolymerizing drugs [26]. TXT at 10^{-8} *M* induced random podosome distribution in Mo-DCs and decreased their adhesive ability, even though TXT at this concentration only slightly impaired a radial microtubule array formation (Fig. 2). Moreover, our data suggest that TXT at concentrations as low as 10^{-9} *M* is able to alter microtubule dynamics and that a functional relationship exists between microtubules, podosomes, migration, and adhesion.

It is also noteworthy that TXT at 10^{-10} *M* reduced IL-12 p40 production from OK-432-stimulated imMo-DCs (Fig. 6). Although our previous data showed that phagocytic ability plays an important role in IL-12 production from OK-432-stimulated imMo-DCs, TXT at concentrations lower than 10^{-7} *M* did not affect phagocytosis of OK-432 by imMo-DCs (data not shown). We also note that TXT at 10^{-7} *M* did not affect IL-12 p40 production of imMo-DCs without OK-432 stimulation. These results underscore the complexity of OK-432-stimulated imMo-DC IL-12 production, which involves antigen capture, antigen processing, and finally IL-12 production.

The ability of TXT at very low concentrations to suppress the motility of DCs is important because only motile DCs are capable of inducing primary sensitization against specific antigens in naive T cells. When patients undergo chemotherapy with taxanes, we should pay more attention to their immunity. TXT-induced immunosuppression may increase the risk of infection in patients. Moreover, in the future, Mo-DCs are likely to be used in immunotherapy in combination with taxanes for treatment of cancer. The possibility that taxane treatment will decrease Mo-DC motility and consequently Mo-DC vaccine efficacy will have to be carefully considered.

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