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The Potential Use of Triterpene Compounds in Dendritic Cells-Based Immunotherapy

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1. Introduction

The immune system is confronted with antigens and proteins that have not been previously encountered by the body. Dendritic cells are professional antigen-presenting cells and play a key role in the induction of these immune responses (Banchereau and Steinman, 1998; Lanzavecchia and Sallustoa, 2001; Mellman and Steinman, 2001). Dendritic cells orchestrate a variety of immune responses by stimulating the differentiation of naïve CD4+ T cells into helper T effectors such as Th1, Th2, Treg cells and Th17 cells and several factors determine the direction of T cell polarization (Romagnani 1994; Kuchroo et al. 1995; Lederer et al. 1996; Tao et al. 1997; Forster et al. 1999; Lezz et al. 1999; Tanaka et al. 2000; O'gara, 20001; Steinman and Dhodapkar, 2001). The cytokine profile present during an immune reaction is an important element in directing the response to T cell polarization. A maturation process, IL-12 production, the up-regulation of MHC and costimulatory molecules, is critical for initiation of primary T cell response. Th1 responses predominate in organ-specific autoimmune disorders, acute allograft rejection and in some chronic inflammatory disorders (Trinchieri and Scott, 1994). Although different dendritic cells subsets may have some intrinsic potential to preferentially induce Th1, Th2, Treg cells or Th17 cells, dendritic cells also display considerable functional plasticity in response to signals from microbes and the local microenvironment (Steinman, 2007). Numerous stimuli can mediate dendritic cells maturation, the best characterized being Toll-like receptor (TLR) ligands and signals such as CD40L delivered by T cells and innate lymphocytes (Hermann et al. 1998). TLRs are expressed mainly on macrophages and dendritic cells, triggering results in the development of effector dendritic cells that promote Th1 responses (Okamoto and Sato, 2003). Recently, several studies proposed the significance of TLR signaling in the induction of anti-cancer immunity. In addition to their essential role in T cell priming, dendritic cells are also involved in innate immunity through the production of cytokines and the activation of NK or NKT cells. Thus, dendritic cells play a pivotal role in orchestrating the immune response.

The hooks of *Uncaria* sp. are contained in Choto-san as the main component herb. Choto-san has been used for hypertension and dementia, and well used as an important of many Chinese prescriptions in China, Korea and Japan. Uncarinic acid (URC) and Ursolic acid are isolated from *Uncaria rhynchophylla* and phytochemically classified as triterpene. A number of alkaloids have been reported as antihypertensive principles from the genus *Uncaria*. URC showed potent inhibitory activity against phospholipase Cγ1 and inhibited the growth of cancer cells at high doses (Lee et al. 2000). Ursolic acid augments the inhibitory effects of anticancer drugs on growth of human tumor cells and triggers apoptosis in cancer cells. Triterpene have been identified as a unique class of natural products possessing diverse biological activities. Terpenes also contain pharmacologically active substance. Recently, we have reported that numerous terpenes induce the differentiation of dendritic cells from human monocytes, and drive Th1 and Th2 differentiation (Takei et al. 2005, 2007, 2008). For immunotherapeutic applications, it appears crucial to identify factors that might affect the differentiation and function of dendritic cells. Although various terpene compounds have pharmacological activity, relatively little is known in regards to the influences URC and Ursolic acid exert on the initiation of specific immune response at the level of dendritic cells. Therefore, to further understand the cellular basis of immunological with abnormalities associated URC and Ursolic acid exposure, we investigated the ability of URC and Ursolic acid on human dendritic cells differentiation (surface molecule), function (cytokines production) and their activation (NF-κB translocation to the nucleus) in detail. Some terpene compounds may lead to the development of effective immunotherapy for cancer.

2. Monocyte-derived dendritic cells phenotype

In order to study the direct effect of URC and Ursolic acid on the function of human monocyte dendritic cells, immature monocyte-derived dendritic cells were exposed to URC and Ursolic acid, and phenotypic and functional dendritic cells maturation was analyzed. URC and Ursolic acid were prepared as previously described (Lee et al. 2000). The purity of URC and Ursolic acid was > 99%. URC and Ursolic acid was dissolved in dimethyl sulfoxide. The concentration of dimethyl sulfoxide in the culture medium was 0.1%, which had no effect on the culture and the production of cytokines under the conditions used in this study. The endotoxin in URC and Ursolic acid was removed using End Trap 5/1 (Profos AG, Regensburg, Germany). Endotoxin levels in URC and Ursolic acid were below 0.05 EU/ml. Human monocytes were cultured with GM-CSF and IL-4 for 6 days under standard conditions, followed by an additional 2 days in the presence of URC and Ursolic acid. Under these conditions, we found that CD1a, CD38, CD40, CD54, CD80, CD83, CD86 and HLA-DR expression levels on URC-primed dendritic cells and Ursolic acid-primed dendritic cells were slightly enhanced. Typical data of phenotypes are shown in Fig.1. The viability of cells treated with a concentration of 0.1 μM URC and 1.0 μM Ursolic acid was >95%. URC and Ursolic acid were kept at 0.1 or 1.0 μM, respectively, for subsequent experiments. As a positive control, human monocytes were cultured with GM-CSF and IL-4 for 6 days, followed by another 2 days in the presence of LPS or TNF-α. LPS and TNF-α are a known dendritic cells maturation-enhancing factors. The expression of co-stimulatory molecules and maturation markers including CD38, CD80, CD83, CD86 and HLA-DR on LPS (100 ng/ml)-primed dendritic cells or TNF-α (25 ng/ml)-primed dendritic cells was higher than URC-primed dendritic cells and Ursolic acid-primed dendritic cells (Fig. 1). Immature dendritic cells (with medium) were generated by cultivating human monocytes with GM-

CSF and IL-4 for 8 days served as a control. The expression level of CD14 as expressed by MFI on day 8 was found to be low or undetectable in some samples. Type 1 interferons' are (IFNs) are important in immune responses against tumors. When human monocyte-derived dendritic cells were stimulated with URC in the presence of IFN-γ (100 ng/ml), the expression of co-stimulatory molecules and maturation markers on URC-primed dendritic cells was significantly increased by IFN-γ administration (data not shown). Immature dendritic cells are efficient in capturing Ag and have a high level of endocytosis. To determine whether mechanisms of Ag capture could also be modulated by URC and Ursolic acid, the endocytic activity was measured in immature dendritic cells, URC-, Ursolic acid-, LPS- and TNF-α-primed dendritic cells. FITC-dextran uptake mediated by URC-, Ursolic acid-, LPS- and TNF-α-primed dendritic cells was lower than immature dendritic cells (data not shown). These results suggested that dendritic cells differentiated by URC and Ursolic acid have down-regulated their endocytic capacity.

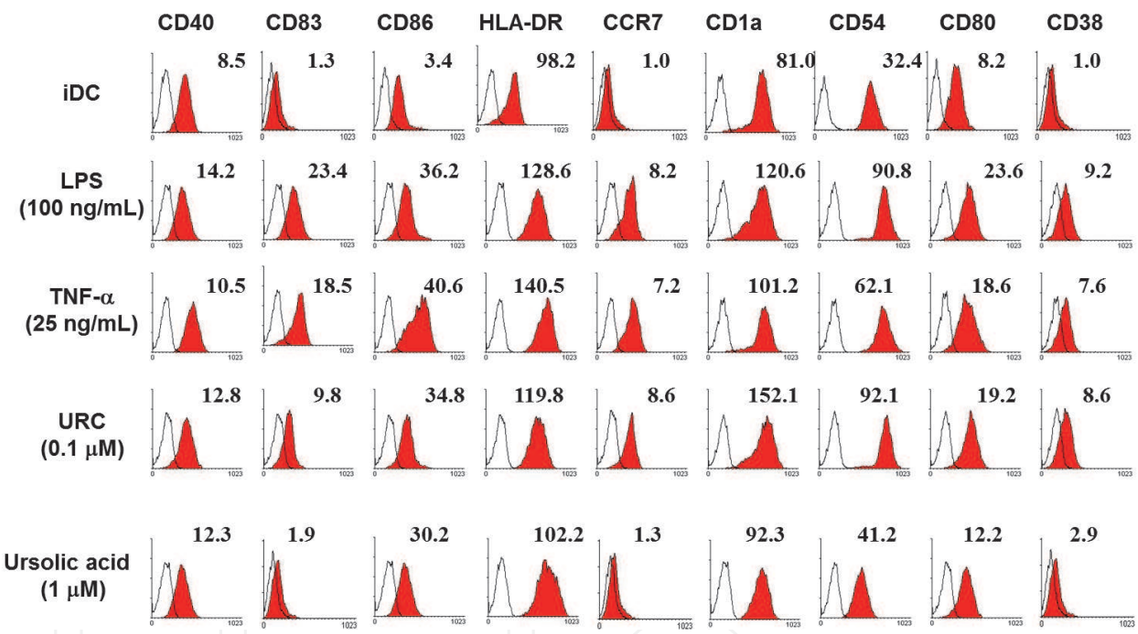


Fig. 1. Phenotype of dendritic cells differentiated with URC, Ursolic acid, LPS or TNF-α. Dendritic cells were generated by stimulating immature dendritic cells with URC (0.1 μM), Ursolic acid (1 μM), LPS (100 ng/ml) or TNF-α (25 ng/ml) and then were stained with FITC-conjugated Ab or PE-conjugated Ab against CD1a, CD38, CD40, CD54, CD80, CD83, CD86, HLA-DR and CCR-7 as described previously (Takei et al. 2005). Antibodies were overlayed with their isotype control. Data are one experiment representative of four independent experiments.

2.1 Immunostimulatory capacity in an allogeneic mixed lymphocyte reaction

The ability to induce allogeneic T cell proliferation is a functional hallmark of dendritic cells *in vitro*. Change in the surface marker expression is also reflected at a functional level, when analyzing the allostimulatory capacity of dendritic cells in an allogeneic mixed lymphocyte reaction. Efficiency in an allogeneic mixed lymphocyte reaction for URC-primed dendritic

cells and Ursolic acid-primed dendritic cells were enhanced in a dose-dependent manner (data not shown). URC-, Ursolic acid- and LPS-primed dendritic cells was demonstrated a higher stimulatory efficiency in an allogeneic mixed lymphocyte reaction than immature dendritic cells and TNF-γ-primed dendritic cells (Fig. 2). Simultaneous dendritic cells stimulation with URC in the presence of IFN-γ (100 ng/ml) appeared to enhance this effect (data not shown). URC–primed dendritic cells and Ursolic acid-primed dendritic cells resulted in enhancement of T cell proliferation, indicating that URC and Ursolic acid potentiate the Ag-presenting activity of dendritic cells in an allogeneic mixed lymphocyte reaction.

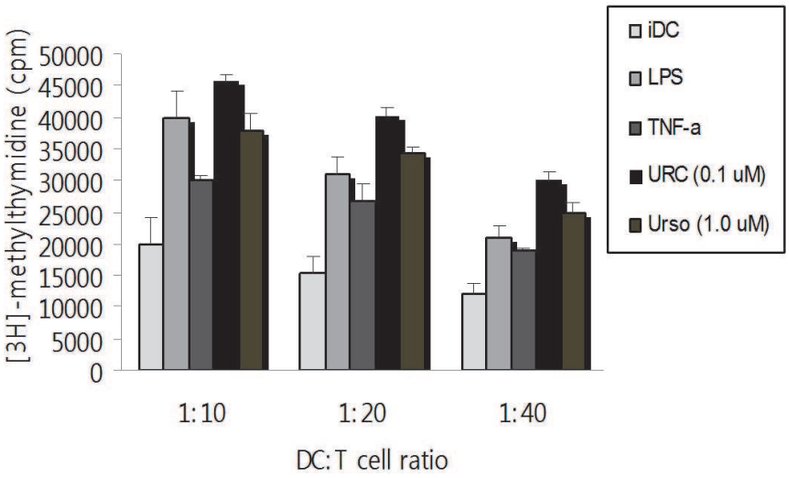


Fig. 2. Allogeneic T cell stimulatory capacity of dendritic cells differentiated with URC, Ursolic acid, LPS or TNF-α. Naïve T cells were co-cultured with gradually increasing doses of dendritic cells and on day 5, [^{3}H] methylthymidine was added 16 h. before measurement of the proliferation responses. Data are the mean cpm ± S.E.M. of five independent experiments. *P< 0.05 compared with immature dendritic cells.

2.2 IL-10 and IL-12p70 release by activated dendritic cells

Since dendritic cells serve as the professional antigen-presenting cells and their secretion of immunoregulatory and proinflammatory cytokines plays a crucial role in T cell priming, we then investigated whether cytokine production by human monocyte-derived dendritic cells was affected by treatment with URC and Ursolic acid. IL-10 is a pleiotropic cytokine known to have inhibitory effects on the accessory functions of dendritic cells and appears to play a central role in preventing overly pathological Th1 or Th2 responses in a variety of settings. In contrast, the level of IL-12 production by myeloid dendritic cells during activation of naïve T cells is a major factor driving the development of Th1 cells. Therefore, we measured IL-10 and IL-12p70 productions in immature dendritic cells (with medium) and in dendritic cells matured for 2 days in the presence of the above factors after stimulation by CD40 L-transfected for 24 h. Measurements of cytokines production were determined by ELISA kit. Major enhancements of IL-12p70 production were caused by URC and Ursolic acid, and this production was dose-dependent manner (Fig.3A and data not shown). Dendritic cells matured with URC in the presence of IFN-γ (100 ng/ml) produced even higher levels of IL-12p70 upon CD40 ligation (data not shown). In contrast, the production of IL-10 by URC-,

Ursolic acid-, LPS- or TNF-α-primed dendritic cells was low. On the other hand, the production of IL-10 and IL-12p70 by immature dendritic cells was low (Fig.3A and B).

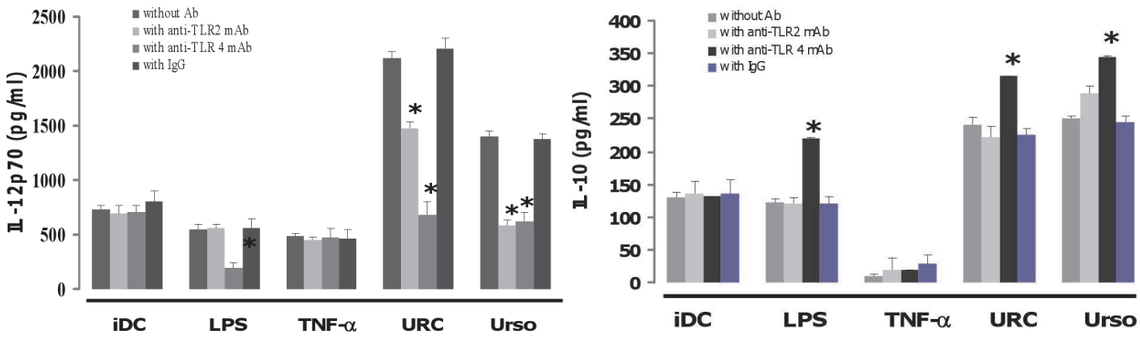


Fig. 3. The production of IL-12p70 by URC-primed dendritic cells and Ursolic acid-primed dendritic cells was inhibited by anti-TLR2 mAb and anti-TLR4 mAb. Dendritic cells were generated by stimulating immature dendritic cells with URC (0.1 μM), Ursolic acid (1 μM), LPS (100 ng/ml) or TNF-α (25 ng/ml). Cells ($4x10^4$ cells/well) were stimulated with the CD40 L-transfected J558 cells ($5x10^4$ cell/well) for 24 h., the production of IL-12p70 (A), and IL-10 (B) was measured by ELISA in culture supernatants. iDC: immature dendritic cells. Data are the mean ± S.E.M. of five independent experiments. *P< 0.05 compared without mAb.

2.2.1 Effects of anti-TLR 2 mAb and anti-TLR4 mAb on cytokine production by URC-primed dendritic cells and Ursolic acid-primed dendritic cells

Dendritic cells activation is mediated by a member of the Toll-like family of receptor and TLR agonists are potent activators of innate immune responses. TLR signaling results in the differentiation of dendritic cells from human monocytes which, in turn, prime an effective Th1 response as recent studies that focused on the molecular mechanisms underlying Th1/Th2 development. We evaluated the role for TLRs in the development of Th1 cells in naïve T cells co-cultured with URC and Ursolic acid. Before stimulation with URC and Ursolic acid, monocyte-derived dendritic cells were incubated in the presence of the TLR2 mAb (10 μg/ml), TLR4 mAb (10 μg/ml) or an isotype control (IgG1). Then, dendritic cells were stimulated with CD40 L –transfected J558 cells for 24 h. The anti-TLR2 mAb and anti-TLR4 mAb inhibited the production of IL-12p70 by URC-primed dendritic cells and Ursolic acid-primed dendritic cells by50-70% (Fig.3A). On the other hand, the production of IL-12p70 by LPS-primed dendrite cells was inhibited by the anti-TLR4 mAb, whereas the anti-TLR2 mAb was not inhibited the production of IL-12p70 by LPS-primed dendritic (Fig.3A). mAb did not inhibit the the production of IL-10 induced by URC-, Ursolic acid- and LPS-primed dendritic cells (Fig.3B). In contrast, the response to TNF-α was not influenced in the presence of the anti-TLR2 mAb and anti-TLR4 mAb did not inhibit (Fig.3A and B). The production of IL-12p70 by URC in combination with IFN-γ was not influenced by adding, whereas the production of IL-10 was not influenced by the anti-TLR4 mAb (data not shown). The production of IL-10 and IL-12p70 by URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells was not influenced when IgG1 was added instead of anti-TLR 2 mAb or anti-TLR4 mAb (Fig.3A and B).

It was left undermined whether TLR2 and TLR4 express on URC-primed dendritic cells and Ursolic acid-primed dendritic cells. To address this question, we examined expression of TLR2 and TLR4 on URC-primed dendritic cells and Ursolic acid-primed dendritic cells. Total cellular RNA was extracted using RNeasy Mini kits (Qiagen GmbH, Germany), according to manufacturer's recommendations. Using RT-PCR, we found that URC-primed dendritic cells and Ursolic acid-primed dendritic cells expressed significant levels of mRNA coding for both TLR2 and TLR4 (data not shown). These data are compatible with URC and Ursolic acid activation of dendritic cells via TLRs.

2.2.2 NF-κB activation and TLR expression

Dendritic cells activation and maturation driven by TLR agonist such as LPS has been clearly associated to NF-κB activation. To determine whether URC and Ursolic acid uses similar activation pathways, we monitored thier ability to activate of the NF-κB translocation into the nucleus. EMES was performed with the Gel Shift assay system (Promega, El, USA). Dendritic cells were cultured in the presence of URC and Ursolic acid for 30 min, 1 h. and 2 h., and nuclear extracts were analyzed for NF-κB content. As shown in Fig.4A, URC was able to induce NF-κB translocation and activation. Similar results were obtained with Ursolic acid-primed dendritic cells and LPS-primed dendritic cells (data not shown and Fig.4A). The expression of TLR2 and TLR4 on monocytes, immature dendritic cells, URC-, Ursolic acid- and LPS-primed dendritic cells was further analyzed by real-time quantitative RT-PCR, because of their different selective expression on dendritic cells. Monocytes and immature dendritic cells expressed the highest levels of TLR2 and TLR4 on the cell population examined. mRNA expression of these two receptors was dramatically down-regulated upon differentiation of monocytes into dendritic cells after 8 days of culture with GM-CSF, IL-4 plus LPS, URC and Ursolic acid (Fig.4B and C). URC-primed dendritic cells and Ursolic acid-primed dendritic cells expressed considerable levels of TLR2 and TLR4. In URC-primed dendritic cells and Ursolic acid-primed dendritic cells, TLR4 expression seemed to be more prominent than TLR2 (Fig.4B and C). The expression of TLR4 on URC-primed dendritic cells and Ursolic acid-primed dendritic cells was higher than that of LPS-primed dendritic cells. Interestingly, we found that upon URC, Ursolic acid and LPS stimulation, mRNA expression of TLR2 and TLR4 was down-regulated with overall mRNA transcript detection levels being lower than in immature dendritic cells. It has been reported that monocyte-derived immature dendritic cells down-regulate TLR2 and TLR4 upon maturation with the corresponding cognate ligand LPS (Ismaili et al. 2002). Our experimental data support their observation showing that LPS in human dendritic cells and monocytes express different mRNA TLR transcripts. One might speculate that URC- and Ursolic acid-induced regulation of TLR2 and TLR4 expression may be involved in positively or negatively modulating the recognition of URC and Ursolic acid motifs by TLR2 and TLR4. URC, Ursolic acid and LPS were, in part, involved in down-regulation of human. These data suggest that URC and Ursolic acid might activate by dendritic cells via a TLR4 and/or TLR2 signaling. Our experiments showed that common pathways are activated by URC, Ursolic acid and LPS, as they modulate TLR expression. An important immunomodulatory property of TLR agonists is their capacity to enhance IL-12 production of dendritic cells and other innate immune cells. TLR signaling frequently generate dendritic cells and enhances the production of IL-12, a major Th-1-inducing cytokine (Okamoto and Sato, 2003). It suggests that dendritic cells activated by TLR2 and TLR4 stimulation may

induce T cell differentiation toward Th1 by presenting antigens to T cells while promoting a Th1-leading situation in the local environment. Ability to promote Th1-type responses plays a key protective role in immunity to tumor. Interestingly, anti-TLR2 mAb and anti-TLR4 mAb enhanced the production of IL-10 by URC-primed dendritic cells and Ursolic acid-primed dendritic cells, and induced T cell differentiation towards Th2. IL-10 can promote Th2 responses that may be inhibitory for Th1 responses and negatively influences the ability of the differentiation of dendritic cells from human monocytes to produce IL-12p70. IL-10 might play a key role in the Th1/Th2 response by inhibiting IL-12p70. Therefore, our results suggest that the development of the Th1/2 response, at least in part, is controlled by the production of IL-12p70 via TLR2 and/or TLR4 signaling on dendritic cells. URC-primed dendritic cells and Ursolic acid-primed dendritic cells provide stronger costimulatory signals and/or the proinflammatory cytokines needed for T cell activation depending on TLR2 and/or TLR4, and induced Th1 development.

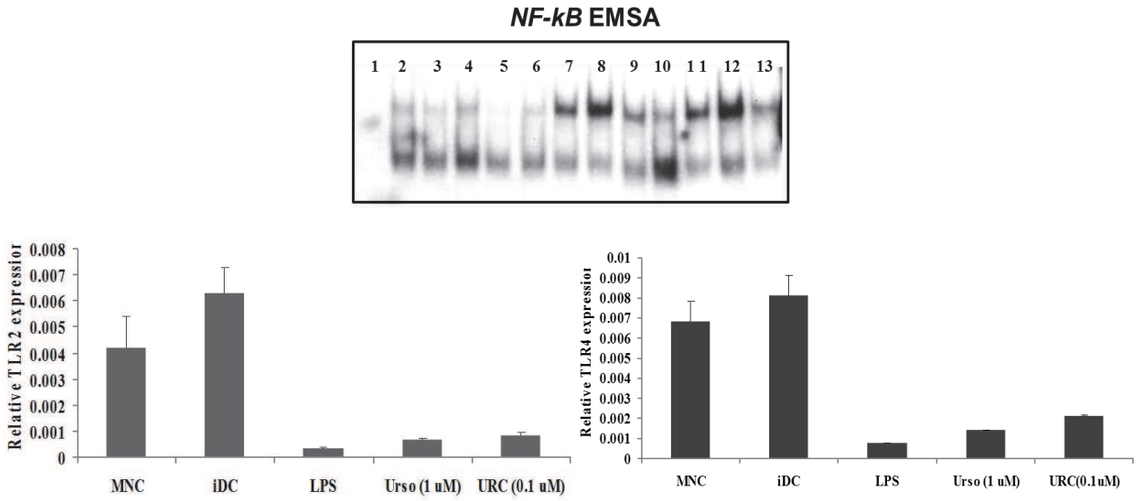


Fig. 4. URC induces NF-κB activation and modulates TLR2 and TLR4. (A) DC were stimulated with URC (0.5 μM), LPS (100 ng/ml) or medium for 30 min, 1 h. and 2 h., and nuclear extracts were analyzed for their NF-κB binding activity using EMSA. 1: Competition (x 100 cold) negative control 2: immature dendritic cells (0 min), 3: immature dendritic cells (30 min) 4: immature dendritic cells (1 h) 5: immature dendritic cells (2 h) 6: LPS (0 min) 7: LPS (30 min) 8: LPS (1 h) 9: LPS (2 h) 10: URC (0 min) 11: URC (30 min) 12: URC (1 h) 13: URC (2 h). (B) Relative TLR expression during the differentiation of dendritic cells. Monocytes were cultured with GM-CSF and IL-4 to differentiate to immature dendritic cells for 8 days. Maturation of dendritic cells was induced by URC (0.1 μM), Ursolic acid (1 μM) or LPS (1 μg/ml). Cells were collected at indicated time; mRNA were extracted and converted to cDNA. The cDNA were subjected to Realtime SYBR Green quantitative PCR using gene-specific primers pair for TLR2, TLR4 and β-actin. Relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method. Left panel: Relative TLR4 expression during differentiation of dendritic cells by LPS in comparison to stimulation of dendritic cells with URC or Ursolic acid. Right panel: Relative TLR2 expression during differentiation of DC by LPS in comparison to stimulation of dendritic cells with URC or Ursolic acid. Data are the mean ± S.E.M. of three independent experiments. *P< 0.05 compared with immature dendritic cells (iDC).

2.3 URC-primed dendritic cells and Ursolic acid-primed dendritic cells promote the differentiation of naïve T cells into Th1 cells

Given that the nature of cytokines secreted by dendritic cells are known to govern the type of T response observed, we evaluated the nature of primary allogeneic T cell responses stimulated by URC-primed dendritic cells and Ursolic acid-primed dendritic cells. Allogeneic URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells induced as substantial increase in the secretion of IFN-γ by T cells (Fig.5A), but had little effect on IL-4 secretion (Fig.5B). Very consistently, the production of IL-4 and IL-10 are coherently dependent on the factors used to drive dendritic cells-maturation. In contrast, the production of IFN-γ and IL-4 induced by naïve T cells co-cultured with immature dendritic cells was low (Fig.5A and B). This Th1 response was confirmed by flow cytometry (data not shown).

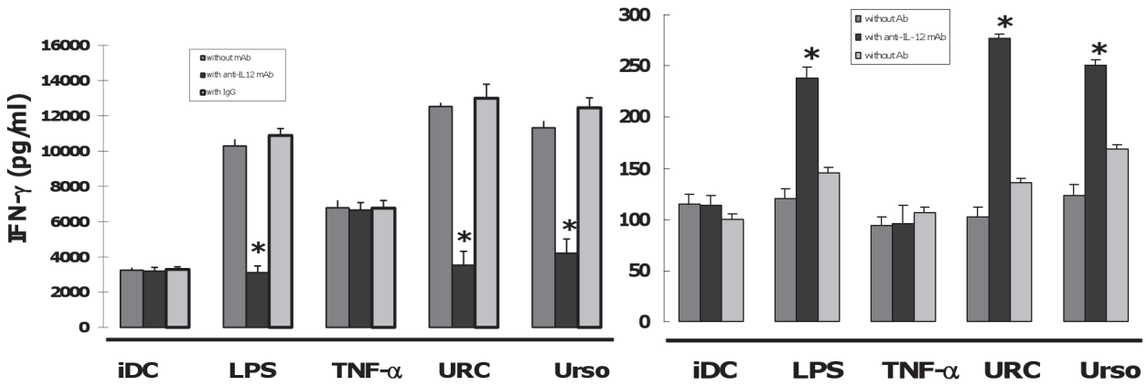


Fig. 5. Effect of anti-IL-12 mAb on polarization of naïve T cells by dendritic cells. Dendritic cells were co-cultured with naïve T cells days in the presence of control Ab or anti-IL-12 mAb (10 μg/ml). After 9 days of expansion in IL-2, T cells were counted and re-stimulated for 24 h with Dynabeads CD3/CD28. After 24 h, IFN-γ (A) or IL-4 (B) was measured by ELISA in culture supernatants. iDC: immature dendritic cells. Data are the mean ± S.E.M. of five independent experiments. *P< 0.05 compared without mAb.

To analyze the contribution of dendritic cells-derived IL-12p70 on the development of Th1 cells, we tested the effect of a neutralizing anti-IL-12 mAb in co-cultures of dendritic cells with naïve T cells. In naïve T cells co-cultured with URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells, neutralization of IL-12 increased the development of IL-4 producing T cells and dramatically decreased the development of IFN-γ producing T cells (Fig.5A and B). In contrast, the production of IFN-γ and IL-4 by naïve T cells co-cultured with URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells was not influenced when IgG1 was used instead of anti-IL-12 mAb (Fig.5A and B). Even higher production of IFN-γ by T cells was induced by dendritic cells matured with URC in the presence of IFN-γ (data not shown). Anti-IL-12 neutralizing Abs decreased IFN-γ secretion, confirming both the presence of bioactive IL-12 and its direct role in IFN-γ induction in this culture system. Th1 and Th2 development depend on the route of immunization, the nature and the concentration of Ag and the balance between IL-4 and IL-12 at priming. Our data showed that LPS-, URC- or Ursolic acid-primed dendritic cells polarized T cells into Th1 via high IL-12p70 secretion upon CD40-L (T cells engagement) stimulation and demonstrated that the production of

IFN-γ by naïve T cells co-cultured with URC-primed dendritic cells and Ursolic acid-primed dendritic cells was affected by the presence of a neutralizing anti-IL-12 mAb. The production of IL-12p70 by dendritic cells is a major inducer of IFN-γ. The reduced induction of IFN-γ after incubation with anti-IL-12 mAb indicates that IFN-γ induction is largely dependent on endogenous IL-12. Therefore, the data suggest that the effect of URC and Ursolic acid on the production of IL-12p70 by dendritic cells and strengthening of the Th1 response by naïve T cells might contribute to a potential antitumor effect of URC and Ursolic acid. The rational for selecting IL-12 production as a potency assay for dendritic cells generated for human therapy is based on IL-12 properties, and its confirmed role in host defense against pathogens and cancer. The generation of effective antitumor immunity involves the production of Th1 cytokines such as IL-12 and IFN-γ that might facilitate the induction and/or activation of tumor Ag-specific CD4 and CD8 cells. IL-12p70 is the cytokine responsible for antitumor responses of T lymphocytes. The understanding of mechanism controlling IL-12 induction by adjuvant in general and URC and Ursolic acid, in particular, may contribute to improving cellular immune responses in human therapies. It seems that TNF-α-primed dendritic cells drive the differentiation of naïve T cells towards Th1 cells via an unknown factor, because TNF-α-primed dendritic cells did not increase the IL-12 production upon adding CD40-L.

2.4 Cytotoxicity of CD8+T cells against T2 target cells

Dendritic cells are professional APC that are required for the initiation of immune responses. In the immunotherapy against malignant diseases, it has been suggested that the induction of tumor antigen-specific CTL is most important for eliminating tumor cells. Antitumor immunity has classically been measured by the quantity of tumor-antigen-specific CD8+ T cells. In this context, it is important to know whether URC-primed dendritic cells and Ursolic acid-primed dendritic cells enhanced specific CTL responses. We compared the CTL responses of autologus CD8+T cells supported by dendritic cells differentiated with URC or TNF-α. In an 8 h. to measure CTL apoptosis by tumor cells, T2 cells loaded with WT-1 peptides strongly induced DNA fragmentation of CTL that were generated with URC-primed dendritic cells pulsed with WT-1 peptides (Fig.6A). Details of the method used in these assay have been described previously (Takei et al. 2004, Mailliard and Lotz 2001, Nakano et al. 1998). As expected from their Th-1-polarizing effect, percentage of DNA fragmentation was dependent on the increased number of CTL cells. Similar results were obtained with a ^{51}Cr release assay to measure lysis of target cells (Fig.6B). URC-primed dendritic cells induced a stronger CTL response than immature dendritic cells or TNF-α-primed dendritic cells. On the other hand, percentage of DNA fragmentation in JAM assay and ^{51}Cr release were low or undetectable when T2 loaded with HIV-1 peptide (unrelated peptide) and T2 (without peptides) were used as the target cells (negative control) (Fig.6A and B). Dendritic cells activated with TLR agonists, especially TLR4 and TLR8 agonists stimulate IL-12-producing human myeloid dendritic cells, which activate CD8 CTL against tumors. Moreover, Kawasaki et al. (2000) have reported that TLR4 mediates LPS-mimetic signal transduction by anti-cancer agents Taxol, a plant-derived diterpene, in mice but not human. More recently, synthetic ligands for TLR4, TLR7 or TLR9 have been through preclinical evaluation and clinical trials against cancer. Therefore, that suggests that URC might be a promising agent for the treatment of cancer. The effects of dendritic cells

matured with Ursolic acid in the CTL responses are not known yet. However, we expect to obtain similar results with URC-primed dendritic cells, because dendritic cells matured with Ursolica cid enhanced the differentiation of naïve T cells towards the Th1 type.

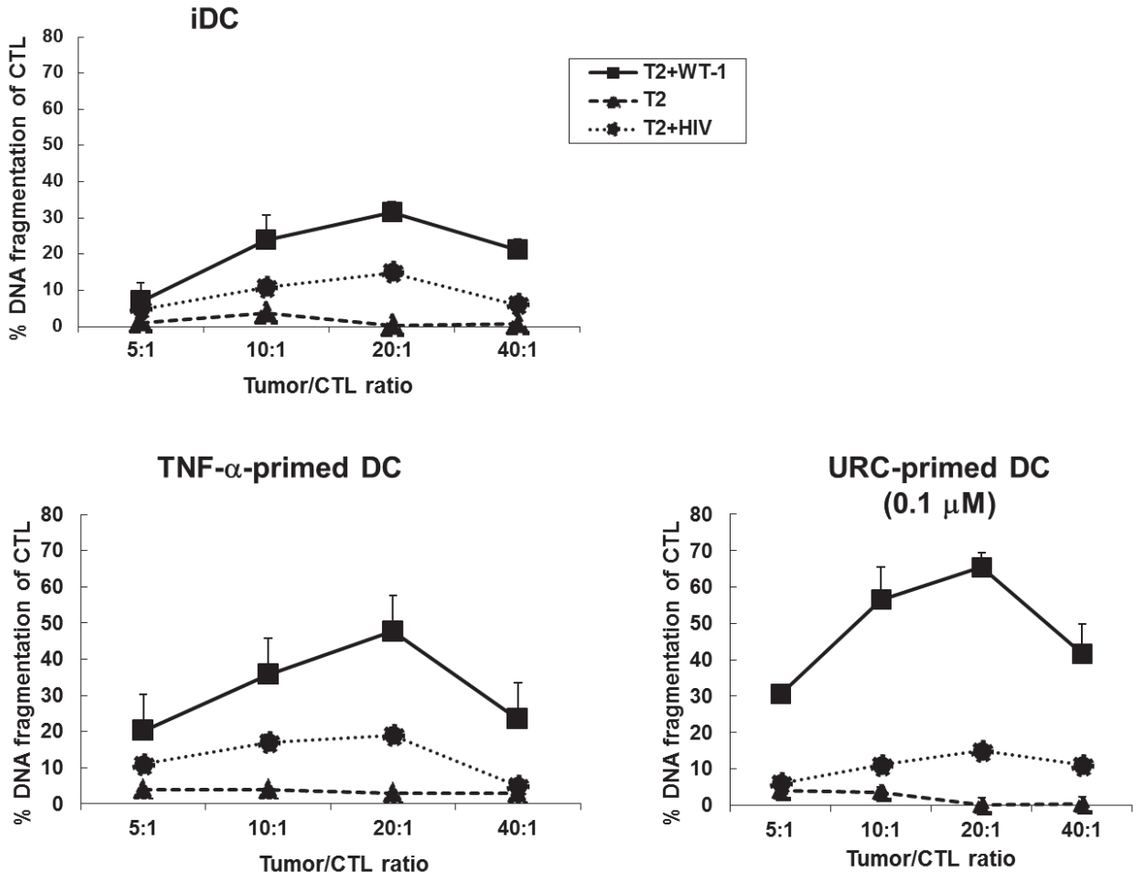


Fig. 6. Autologus CD8+T cells incubated with URC-primed dendritic cells showed higher cytolytic activity against T2 target cells loaded with WT-1 peptide at a high effector-to-target ratio than against T2 target cell without WT-1 peptide. (A) The CTL were labeled with [^{3}H]-methylthymidine and served as the target for tumor cell lines. T2 cells induced more DNA fragmentation of CTL that were generated with URC-primed dendritic cells pulsed with WT-1 peptides than those that were generated with immature dendritic cells or TNF-α-primed DC pulsed with WT-1 peptides. (B) Specific lysis was measured by ^{51}Cr release assay. Data are the mean ± S.E.M. of three independent experiments. *P< 0.05 compared with T2.

2.5 URC-primed dendritic cells and Ursolic acid-primed dendritic cells are capable of migration *in vitro*

The ability of dendritic cells to migrate to local lymph nodes and their subsequent presentation of antigen to T cells play an essential role in the initiation of adaptive immunity. In tissues, mature dendritic cells must be responsive to lymph node derived signals, but must also be able to down-regulate tissue anchoring proteins including E-

cadherin that would otherwise detrimental dendritic cells migration and antigen presentation to naïve T cells. Migration to the secondary lymphoid organs and subsequent antigen presentation requires dendritic cells maturation, a process that is associated with up-regulation of co-stimulatory molecules. We investigated the migratory capacity of URC-primed dendritic cells and Ursolic acid-primed dendritic cells toward CCL19 and CCL21. URC-primed dendritic cells and Ursolic acid-primed dendritic cells had migration in response to CCL19 and CCL21 (Fig.7), and slightly up-regulated the expression of CCR7 and CD38 on URC-primed dendritic cells and Ursolic acid-primed dendritic cells (Fig.1). Expression of CCR7 seems also to be important for other aspects of dendritic cells biology, in particular in enhancing chemotaxis and trans-endothelial passage in response to CCL19 and CCL21. Recently, it has been reported that expression of CD38 on dendritic cells is essential for their coordinated migration to the T cell area of draining lymph node and increase dendritic cells function (Trepiakas et al. 2009). These results suggest that URC-primed dendritic cells and Ursolic acid-primed dendritic cells migrate *in vivo* and is a promising approach for the treatment of cancer. In most clinical trials using dendritic cells-based immunotherapy, immature monocyte-derived dendritic cells pulsed with tumor antigen peptides were used. Recent studies showed that mature dendritic cells could be a better antitumor adjuvant. However, there is no clear answer yet.

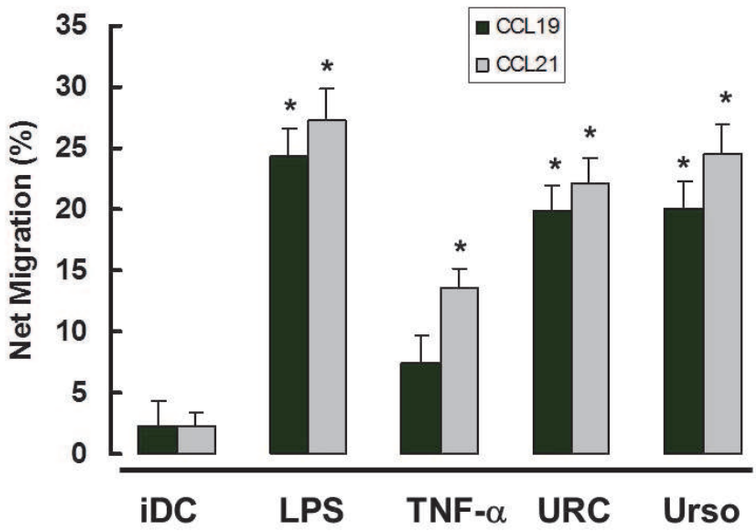


Fig. 7. Chemotaxis in response to CCL19 and CCL21 by URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells. URC-, Ursolic acid-, LPS- or TNF-α-primed dendritic cells were prepared and recovered, and their migratory abilities in response to CCL19 (500 ng/ml) and CCL21 (500 ng/ml) were determined *in vitro*. Data are the mean ± S.E.M. of three independent experiments. *P< 0.05 compared with immature dendritic cells (iDC).

3. In conclusion

We described in this chapter a very promising and cost effective dendritic cells maturation factor consisting of URC and Ursolic acid, both mature dendritic cells which meet the dendritic cells criteria important for efficient immunotherapy, including the capacity to migrate and the production of IL-12p70 upon CD40 triggering. Although based on *in vitro*

results, URC and Ursolica cid may be used in dendritic cells-based vaccine for cancer immunotherapy. Several mechanisms have been proposed to explain the apparent adjuvant effects of TLR agonists on atitumor immunity. TLR trigger the secretion of critical cytokines, and TLR can stimulate the proliferation of CD4+ T cells and CD8+ T cells. Moreover, TLR signaling frequently enhances the production of IL-12 in dendritic cells. Thus, it is strongly suggested that dendritic cells matured with TLR stimulation may induce T cell differentiation toward Th1 by presenting antigens to T cells while promoting a Th1-leading situation in the local environment. Several TLR agonists have been developed as anticancer drugs. We expect to be able to correlate the in vitro dendritic cells product attributes with *in vivo* immunologic and clinical end point.

4. Acknowledgments

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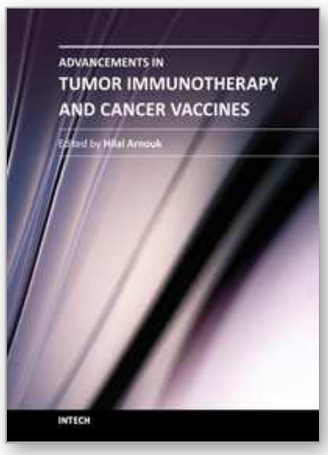
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Harnessing the potential of the human body's own immune system to attack malignant tumor cells has been the goal of many scientific investigators in recent years, with advances in cancer biology and immunology enabling cancer immunotherapy to become a reality. World-class bench and clinical researchers have joined forces to collaborate and review current developments and trends in cancer immunology for the purposes of this book, and the result is a promising review of contemporary clinical treatments. In each chapter the authors present the scientific basis behind such therapeutic approaches, including cancer vaccines with special focus on prostate cancer, melanoma and novel approaches utilizing both innate and adaptive immune responses.

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