

Classification of Chemotherapeutic Agents Based on Their Differential *In vitro* Effects on Dendritic Cells

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Abstract

Despite the crucial roles dendritic cells (DC) play in host immunity against cancer, the pharmacologic effects of many chemotherapeutic agents have remained mostly unknown. We recently developed a DC biosensor clone by engineering the stable murine DC line XS106 to express the yellow fluorescent protein (YFP) gene under the control of interleukin (IL)-1 β promoter. In this study, the resulting XS106 pIL1-YFP DC clone was used to screen 54 anticancer drugs. Each drug was tested at five concentrations (0.1–10 μ mol/L) for its effects on YFP expression, cell viability, and granulocyte macrophage colony-stimulating factor–dependent growth. Our unbiased systematic screening unveiled a striking heterogeneity among the tested anticancer drugs in their effects on the three functional variables. Interestingly, 15 drugs induced significant YFP expression at subcytotoxic concentrations and were thus categorized as “DC-stimulatory” anticancer drugs. These drugs were subsequently found to induce at least one of the characteristic maturational changes in mouse bone marrow–derived DCs. For example, vinblastine, a prototypic drug of this class, induced the production of IL-1 β , IL-6, and IL-12, increased surface expression of CD40, CD80, CD86, and MHC class II, and augmented T cell–stimulatory capacity of DCs. Not only do these results illustrate the differential pharmacologic effects of commonly used chemotherapeutic agents on DCs, they may also provide a conceptual framework for rationale-based selection and combination of anticancer drugs for clinical application. [Cancer Res 2009;69(17):6978–86]

Introduction

Dendritic cells (DC) possess all functional properties required for presenting tumor-associated antigens to effector T cells, and thereby, protecting the host from tumor development (1). However, tumor cells may escape from immune surveillance and even shape their immunologic phenotype via cancer immunoediting (2, 3). For example, cancer cells (and tumor-infiltrating leukocytes) suppress the development/maturation of DCs by elaborating vascular endothelial growth factor, transforming growth factor- β , interleukin (IL)-10, and prostaglandin E₂ (3, 4). This problem may be overcome by DC-targeted immunotherapeutic approaches, such as

adoptive transfer of *ex vivo* expanded DCs after loading with tumor antigens, intratumor administration of DC-attracting chemokines, and use of DC-stimulatory adjuvants (5, 6). Tissue-resident DCs play immunosurveillance roles in the steady state by sampling materials and detecting aberrant signals, including microbial products and proinflammatory mediators. Upon sensing such pathologic signals, DCs increased the surface expression of MHC II and costimulatory molecules, elaborate cytokines and chemokines, heighten the ability to activate immunologically naïve T cells, and migrate to draining lymph nodes. Those DCs that have completed these changes (termed mature DCs) are fully capable of inducing adaptive immune responses, whereas immature DCs are involved in immunologic tolerance (7).

Chemotherapy remains the standard treatment modality for many advanced cancers, although it is neither curative as a stand-alone protocol nor effective in augmenting host immune responses to cancer cells. Instead, most chemotherapeutic agents are likely to impair the clonal expansion of effector lymphocytes as well as homeostasis of innate leukocytes, thereby potentially suppressing host immunity (8). This is not totally unexpected because classic chemotherapeutic agents were originally discovered based on their activities to interrupt with metabolic processes for DNA, RNA, and protein biosynthesis. It has recently become evident that certain chemotherapeutics augment host immunity (9). For example, selected agents increase the immunogenicity of cancer cells (10, 11), preferentially inhibit the function of regulatory T cells (12, 13), or elicit macrophage activation (14). Few systematic studies have been reported in the literature comparing diverse anticancer agents for their effects on DCs. Thus, we sought to fill this apparent gap of knowledge by conducting an unbiased screening of a broad spectrum of chemotherapeutic agents. To achieve this time-efficiently and cost-efficiently, we used the recently developed DC biosensor system. Based on the observation that DC maturation is accompanied by rapid and robust IL-1 β mRNA expression (15), we engineered the murine DC line XS106 to express the yellow fluorescent protein (YFP) gene under the control of IL-1 β promoter. The resulting XS106-pIL1-YFP DC clone exhibited strong YFP fluorescent signals upon exposure to all tested agents known to induce DC maturation (15). Here, we report the differential effects of 54 anticancer drugs on DC maturation, survival, and growth.

Materials and Methods

Cell lines. The XS106-pIL1-YFP DC clone (15) and the parental XS106 DC line were maintained in complete RPMI 1640 supplemented with 0.5 ng/mL of granulocyte macrophage colony-stimulating factor (GM-CSF) and 5% NS cell–conditioned medium (16).

Reagents. Anticancer drugs with diverse chemical structures and mechanisms of action (17) were purchased from commercial vendors (Supplementary Table S1). All the drugs were dissolved in DMSO at 2 mmol/L and tested at different concentrations with a final DMSO

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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concentration of 0.5%. Ovalbumin (OVA; Sigma) was dissolved in PBS at 100 mg/mL and then passed through the polymyxin B column (Pierce) repeatedly until endotoxin became undetectable by the QCL-1000 system (Cambrex Bio Science).

Classification of anticancer drugs. The DC biosensor clone was incubated in 96-well plates (5×10^4 cells/200 μ L/well) for 16 h with each drug at five concentrations in triplicate and then examined for YFP expression and propidium iodide (PI) uptake (15). The parental XS106 DC line was cultured in 96-well plates with 0.5 ng/mL of GM-CSF in the presence of each test drug and examined for 3 H-thymidine uptake on day 2. Regression curves were generated from the dose-response data sets for YFP expression, growth inhibition, and cytotoxicity using the Regression Wizard (Sigmoidal dose-response) function in the Sigma Plot program to calculate the “minimal effective doses” (MED). The 54 drugs were clustered based on the MED values for the three functional variables using the Gene Tree function in the GeneSpring program.

Effects of type 1 drugs on DC phenotype and function. Bone marrow (BM)-derived DC cultures were propagated as before (18, 19). After 24 h of incubation with each test drug at 10^6 cells/mL, the cells were examined for surface phenotype within the CD11c⁺ populations and release of cytokines by ELISA. Following 24-h incubation with a test drug, BM-DCs were incubated for 10 min with 5 mg/mL of FITC-conjugated dextran (FITC-DX, 70 kDa; Sigma) at 4°C or 37°C, washed extensively, and then examined for FITC signals by CD11c⁺ cells. After drug pretreatment, BM-DC preparations (derived from BALB/c mice) were washed thrice and then cocultured in 96-well round-bottomed plates with splenic T cells purified from C57BL/6 mice (5×10^4 cells/well). The magnitude of T cell proliferation was assessed by 3 H-thymidine uptake on day 4 (15). To test antigen cross-presentation, vinblastine-pretreated BM-DCs (derived from C57BL/6 mice) were pulsed with OVA protein (0.3 mg/mL) or OVA_{257–264} peptide (1 μ g/mL) for 1 h, washed extensively, and added to microcultures of CD8 T cells (5×10^4 cells/well) freshly purified from OT-I transgenic mice. In some experiments, BM-DCs derived from the IA β -enhanced green fluorescent protein (EGFP) knock-in mice (20) were cocultured with vinblastine-pretreated BM-DCs from wild-type mice. Human DC cultures derived from the CD34⁺ cord blood progenitors were purchased from Mat Tek Corporation (21).

Statistical analysis. Each test drug was compared with vehicle alone control by a two-tailed Student's *t* test. All experiments were repeated at least thrice to assess reproducibility.

Online supplemental material. Supplementary Fig. S1 (vinblastine-induced phenotypic maturation of human DCs) and Supplementary Table S1 (drugs screened in this study and their MED values).

Results

Differential effects of anticancer drugs on DCs. From commonly used chemotherapeutic agents (17), we constructed a test library consisting of 12 topoisomerase inhibitors, 5 inhibitors of microtubule polymerization, 14 alkylating agents, 8 antimetabolites, 4 platinum agents, 4 hormonal agents, and 7 others (Supplementary Table S1). Each of the 54 drugs were added at five different concentrations (0.1–10 μ mol/L) to microcultures of the DC biosensor clone in triplicate. After 16 hours of incubation, we examined YFP expression as an indicator of DC maturation (*red lines*, Fig. 1A). Several drugs induced significant YFP expression in a dose-dependent manner. They included camptothecin sodium salt (CPT-Na), which was identified as a “hit” compound in our previous DC biosensor-based screening of a small compound library from the National Cancer Institute (15), as well as other topoisomerase inhibitors, such as doxorubicin (ADR), daunorubicin (DNR), epirubicin (EPR), idarubicin (IDR), mitoxantrone (MXT), and ellipticine (ELP). YFP expression was also triggered by antimicrotubule agents, including vinblastine (VBL), paclitaxel (PTX), and docetaxel (DCT). Two alkylating agents,

mechlorethamine (HN2) and diaziquone (AZQ), and an antimetabolite, cladribine (2-CdA), also induced YFP expression.

The same 54 drugs were also tested for their cytotoxicity by measuring PI uptake by the DC biosensor clone (*green lines*, Fig. 1A). Varying degrees of cytotoxicity were observed with all tested topoisomerase inhibitors, except for irinotecan (CPT-11) and etoposide (VP-16). Antimicrotubule inhibitors, VBL, PTX, vincristine (VCR), and vinorelbine (VRL), and a retinoid, alitretinoin (9-cRA), also induced modest reduction in cell viability. Other drugs exhibited significant cytotoxicity in the tested concentration range.

As the third readout, we measured the effect on parental XS106 DC growth primarily to validate the biological activities of the tested drugs (*blue lines*, Fig. 1A). A total of 35 drugs, including all tested topoisomerase inhibitors and microtubule inhibitors, inhibited GM-CSF-dependent growth of XS106 DCs in a dose-dependent fashion.

Classification of anticancer drugs based on their differential effect on DCs. Our screening results unveiled a striking diversity among the tested anticancer drugs in their effects on maturation, survival, and growth of the XS106 DCs. To interpret the data systematically, we calculated the MED value for DC maturation from each dose-dependence curve, which was defined as the concentration required for producing 30% increase in YFP signals above the baseline level. Likewise, we calculated the MED values for DC killing and for DC growth arrest, defined as the concentration causing 77% (i.e., 100/130) reductions in cell viability and 3 H-thymidine uptake, respectively. The actual MED values of all drugs tested are described in Supplementary Table S1.

We next used the MED values as denominators to categorize the drugs based on their relative efficiencies to induce DC maturation, DC killing, and DC growth arrest (Fig. 1B). This approach enabled us to classify the 54 chemotherapeutic agents into (a) type 1 drugs (15 agents, *red lines* Fig. 1B) that delivered DC maturation signals at concentrations causing only marginal DC death, (b) type 2 drugs (19 agents, *blue lines*) that primarily inhibited DC growth, (c) type 3 drug (actinomycin D or AMD, *purple line*) that caused DC growth arrest and DC death without delivering DC maturation signals, and (d) type 0 drugs (19 agents, *black lines*) that cause no substantial change at the tested concentrations. Anticancer drugs categorized as type 1 were of particular interest because some of them might be used as “immunostimulatory” chemotherapeutics—they included nine topoisomerase inhibitors (CPT, CPT-Na, ADR, DNR, EPR, IDR, VP-16, MXT, and ELP), three antimicrotubule agents (VBL, PTX, and DCT), two alkylating agents (HN2 and AZQ), and a purine analogue (2-CdA).

Do type 1 anticancer drugs indeed induce DC maturation?

Although the DC biosensor system provides a time-efficient and cost-efficient assay platform, it has two major limitations. First, the indicator we used (i.e., IL-1 β promoter activation) may not fully reflect the state of DC maturation. Second, the DC preparation we examined (i.e., XS106 DC line) may not fully represent bona fide DCs. In fact, the magnitude of YFP expression inducible by a given stimulus did not correlate with its actual effect on BM-DC preparations (15). We next sought to determine whether some of the type 1 anticancer drugs would induce characteristic changes known to accompany DC maturation. In this regard, we observed previously that CPT-Na, which was identified as a hit from the National Cancer Institute small compound library, induces phenotypic maturation of murine BM-DCs without causing robust cytokine production or augmenting their T cell-stimulatory capacity (15). Thus, we tested the remaining 14 type 1 drugs in

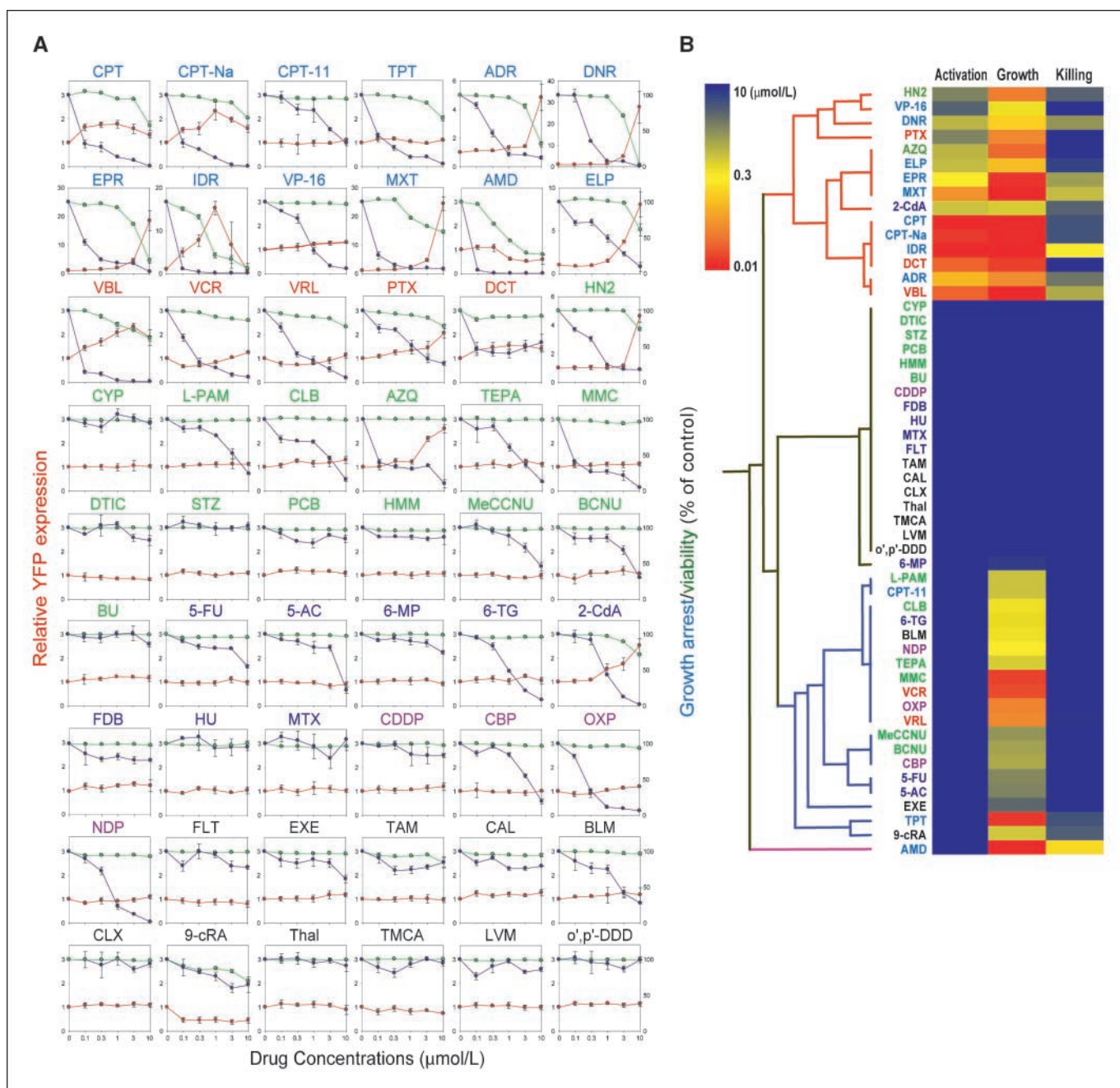


Figure 1. Classification of anticancer drugs based on their differential effects on DCs. **A**, each drug was tested at five different doses (0.1–10 $\mu\text{mol/L}$) for its effectiveness in elevating YFP expression by the DC biosensor clone (red lines), to kill the same DC clone (green lines), and to inhibit GM-CSF-dependent growth of the parental XS106 DC line (blue lines). Points, mean from three independent experiments; bars, SD ($n = 3$). Note that YFP expression levels are shown in different scales for some drugs. **B**, from the above dose-dependency curves, the MED values were determined for DC maturation (YFP expression), growth arrest (^3H -thymidine uptake), and killing (PI uptake). These values, converted into different colors as indicated in the color scale, were then used as quantitative denominators for clustering the drugs. Mechanisms of action for individual drugs are indicated in different colors as shown in Supplementary Table S1.

this study for their effects on BM-DCs. Each drug was tested at a predetermined concentration, i.e., the highest MED value for DC maturation selected from three independent screening experiments. DNR and HN2, which were found to kill large numbers of BM-DCs at the above defined concentrations, were tested at arbitrarily chosen concentrations of 0.3 and 0.6 $\mu\text{mol/L}$, respectively.

BM-DCs were cultured for 24 hours with each test drug and then examined for surface expression of CD40, CD80, CD86, and MHC II.

A microtubule inhibitor, vinblastine, seemed to increase the expression of all four markers of DC maturation, without affecting cell viability measured by PI uptake (Fig. 2A). We did the experiment with triplicate samples and scored the observed difference as “up-regulation” only when a given drug induced a statistically significant ($P < 0.05$) and biologically substantial ($>30\%$) increase in the median fluorescence intensity value above the baseline level. Three drugs, VBL, PTX, and VP-16, fulfilled the dual criteria for up-regulating all four of the phenotypic markers

for DC maturation (red, Fig. 3A). Other drugs that were found to up-regulate CD40 expression (CPT, ADR, IDR, ELP, HN2, and AZQ) failed to increase the expression of CD80, CD86, or MHC II. The viability of BM-DCs remained mostly unchanged after 24 hours of incubation with any drug at the tested concentration.

Supernatants collected from the above BM-DC cultures were then examined for IL-1 β , IL-6, IL-12 p40, and tumor necrosis factor- α (TNF α ; Fig. 3B). Once again, we used the same dual criteria to define a statistically significant ($P < 0.05$) and biologically substantial (>30%) change in cytokine production. Nine drugs induced up-regulated production of IL-1 β protein by BM-DCs, and two down-regulated IL-1 β production. Likewise, production of other cytokines was up-regulated by some of the type 1 drugs. Interestingly, mitoxantrone, which failed to induce phenotypic maturation, up-regulated the production of all tested cytokines. Vinblastine, which induced full phenotypic maturation, up-regulated the production of IL-1 β , IL-6, and IL-12, whereas down-regulating TNF α production. These findings further illustrated the differential effects of type 1 drugs on different functional variables of DC maturation.

The most crucial functional property of mature DCs is the ability to activate immunologically naïve T cells efficiently. Thus, we

pretreated BM-DCs with each type 1 drug and then tested their efficiency to activate allogeneic T cells. DCs pretreated with CPT, ELP, VBL, PTX, DCT, HN2, AZQ, or 2-CdA exhibited significantly ($P < 0.01$) augmented T cell-stimulatory capacity (Fig. 2B). By contrast, DCs pretreated with daunorubicin showed a significantly reduced ability to activate allogeneic T cells. The remaining type 1 drugs (ADR, EPR, IDR, VP-16, and MXT) induced no detectable changes in the T cell-stimulatory potential.

In summary, not all type 1 anticancer drugs uniformly induced a full spectrum of maturational changes in DCs (Fig. 4). Instead, we observed marked heterogeneity in their abilities to elevate the surface expressions of CD40, CD80, CD86, and MHC II to trigger the production of IL-1 β , IL-6, IL-12, and TNF α , and to augment the T cell-stimulatory capacity of DCs. It should be noted here that each drug produced at least one of these characteristic changes. Most notably, vinblastine was found to produce all the maturational changes of DCs (except for TNF α production) in BM-DCs.

Pharmacologic effects of vinblastine on DCs. Having identified vinblastine as a prototypic type 1 anticancer drug, we next further characterized its effects on DCs. In dose-dependency experiments, vinblastine ranging from 0.1 to 1.0 $\mu\text{mol/L}$ induced IL-1 β , IL-6, and IL-12 p40 production and elevated CD40, CD80,

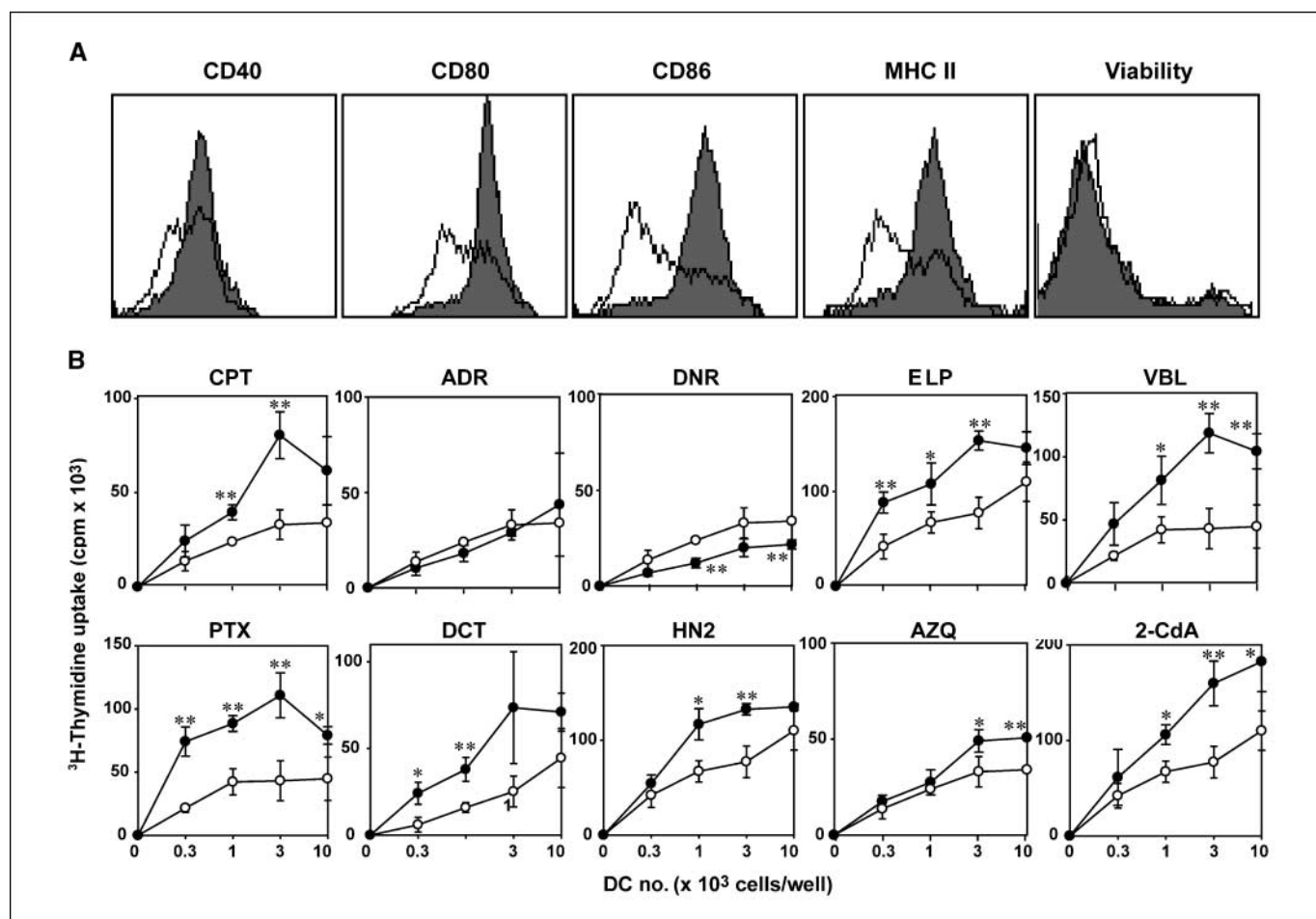


Figure 2. Effects of vinblastine and other type 1 anticancer drugs on BM-DCs. A, BM-DCs were incubated for 24 h with 0.3 $\mu\text{mol/L}$ of vinblastine (filled histograms) or vehicle alone (open histograms) and then examined for surface expression of the indicated markers and for PI uptake within the CD11c $^+$ populations. B, BM-DCs (from BALB/c mice) were incubated for 24 h with the indicated drugs (●) or vehicle alone (○). After extensive washing, the DC preparations were cultured at the indicated numbers with T cells purified from C57BL/6 mice. Points, means of ^3H -thymidine uptake; bars, SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$, statistically significant differences compared with the vehicle-treated control.

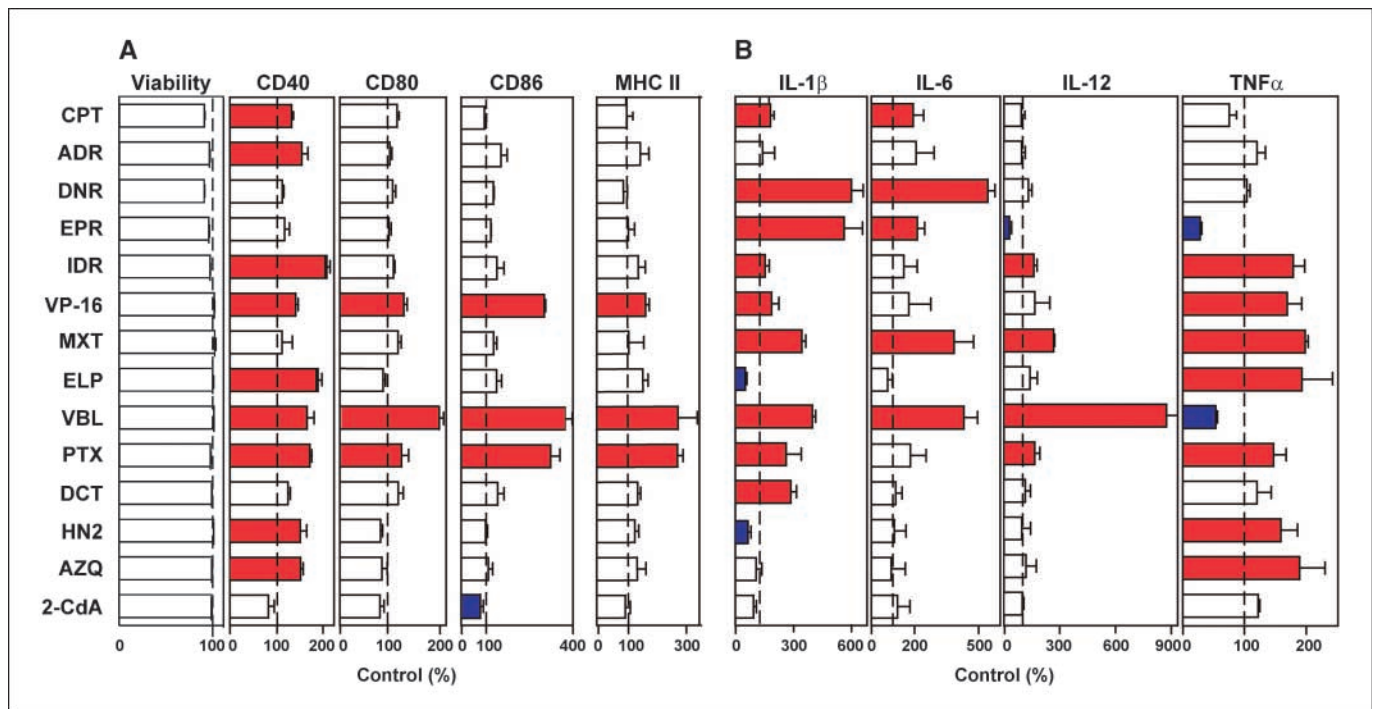


Figure 3. Relative efficacy of type 1 anticancer drugs to promote phenotypic maturation and cytokine production by BM-DCs. *A*, BM-DCs were incubated for 24 h with each drug or vehicle alone in triplicate and then examined for surface expression of the indicated markers and for PI uptake within the CD11c⁺ populations. *B*, supernatants harvested from the same cultures were examined for the indicated cytokines. Broken lines, baseline values observed after treatment with vehicle alone. Statistically significant ($P < 0.05$) and biologically relevant (>30%) increase above (red) or decrease below (blue) the baseline value.

CD86, and MHC II expression (Fig. 5A). To begin to understand the mechanisms by which vinblastine induces DC maturation, we determined whether vinblastine-pretreated BM-DCs (from wild-type mice) might indirectly activate secondary BM-DC preparations (derived from the IAβ-EGFP knock-in mice). The EGFP/

CD11c⁺ DC populations showed CD86 up-regulation after coculturing with vinblastine-pretreated DCs (Fig. 5B), and the observed CD86 expression was up-regulated further by adding vinblastine directly to the cocultures of vinblastine-pretreated DCs and nontreated DCs (Fig. 5C). These results suggest dual mechanisms

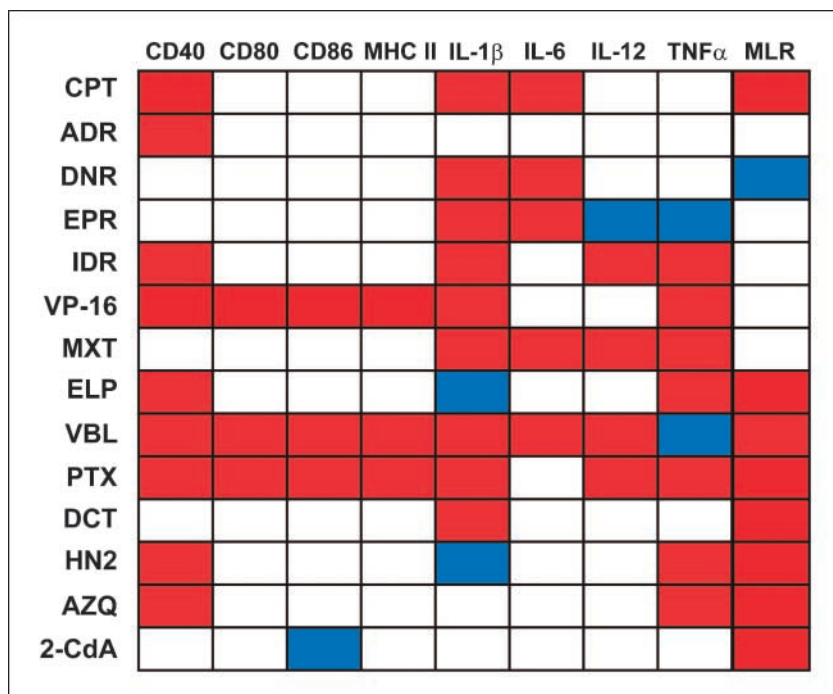


Figure 4. Summary of differential effects of type 1 anticancer drugs on BM-DCs. Effects of the tested type 1 anticancer drugs on different phenotypic and functional variables of DC maturation are summarized in the checkerboard. Statistically significant and biologically relevant increases (red) or decreases (blue).

of vinblastine action, i.e., direct and indirect DC activation. With regard to mechanisms for the indirect pathway, the cytokines being released from vinblastine-pretreated DCs (Fig. 3B) may play functional roles.

Although it is generally believed that DCs efficiently incorporate exogenous antigens only in the immature state (22), West and colleagues showed that BM-DCs exhibit augmented uptake of FITC-DX following stimulation with various toll-like receptor

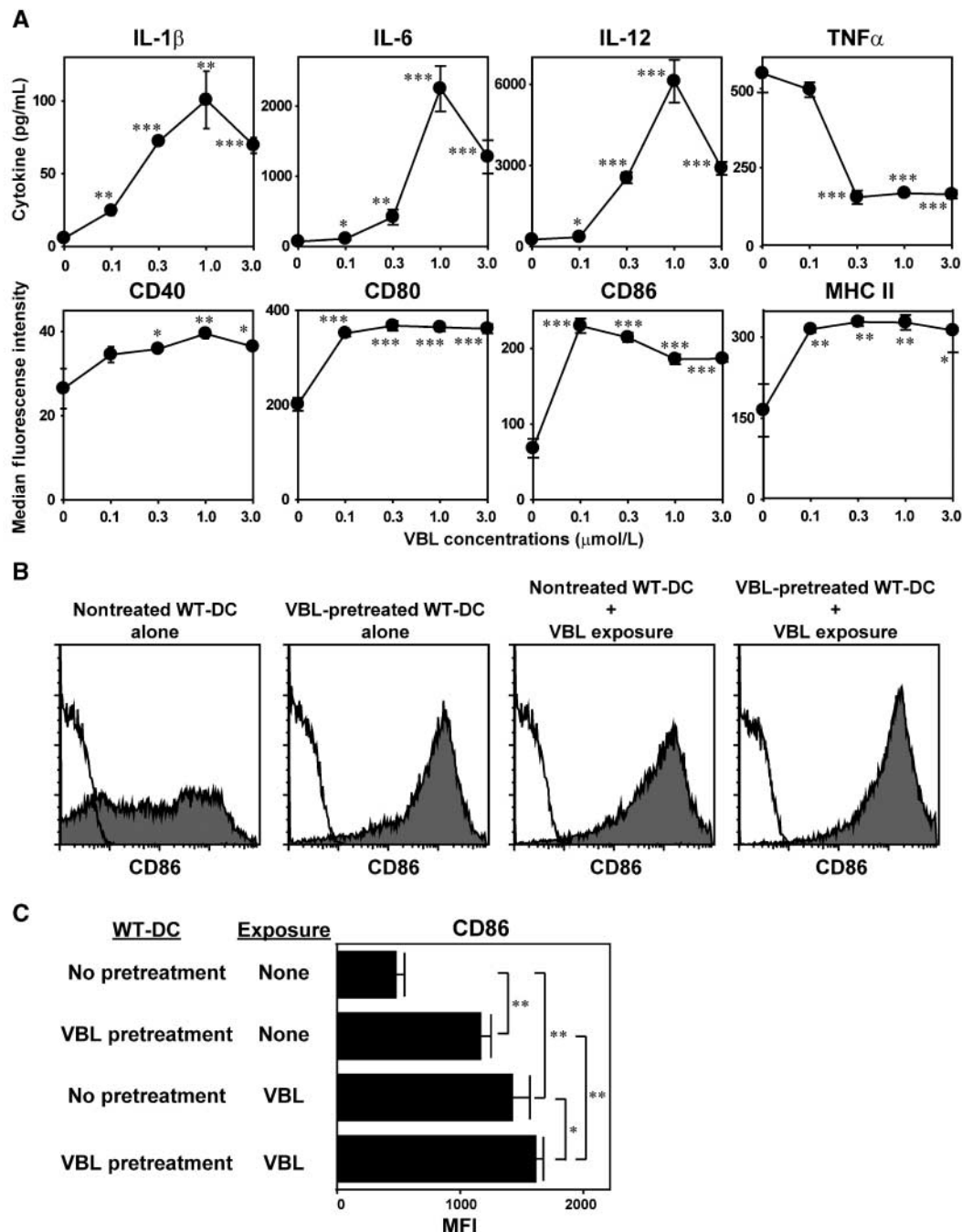


Figure 5. Dose-dependency and mechanisms of vinblastine-induced DC maturation. *A*, BM-DCs were incubated for 24 h with vinblastine at the indicated concentrations and then examined for the production of the indicated cytokines and expression of the indicated markers. *Points*, mean of cytokine concentrations and median fluorescence intensities; *bars*, SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, statistically significant differences compared with the vehicle-treated control. *B*, BM-DCs derived from wild-type mice were preincubated for 24 h with 1 μ mol/L of vinblastine or vehicle alone, washed extensively, and added to cultures of BM-DCs derived from the IA β -EGFP knock-in mice. After an additional 24 h of incubation in the presence or absence of freshly added vinblastine (1 μ mol/L), the EGFP/CD11c $^{+}$ DC populations were analyzed after staining with anti-CD86 monoclonal antibody (*filled histograms*) or an isotype-matched control IgG (*open histograms*). *C*, CD86 expression levels in the above experiment were compared among four experimental conditions ($n = 3$; *, $P < 0.05$; **, $P < 0.01$).

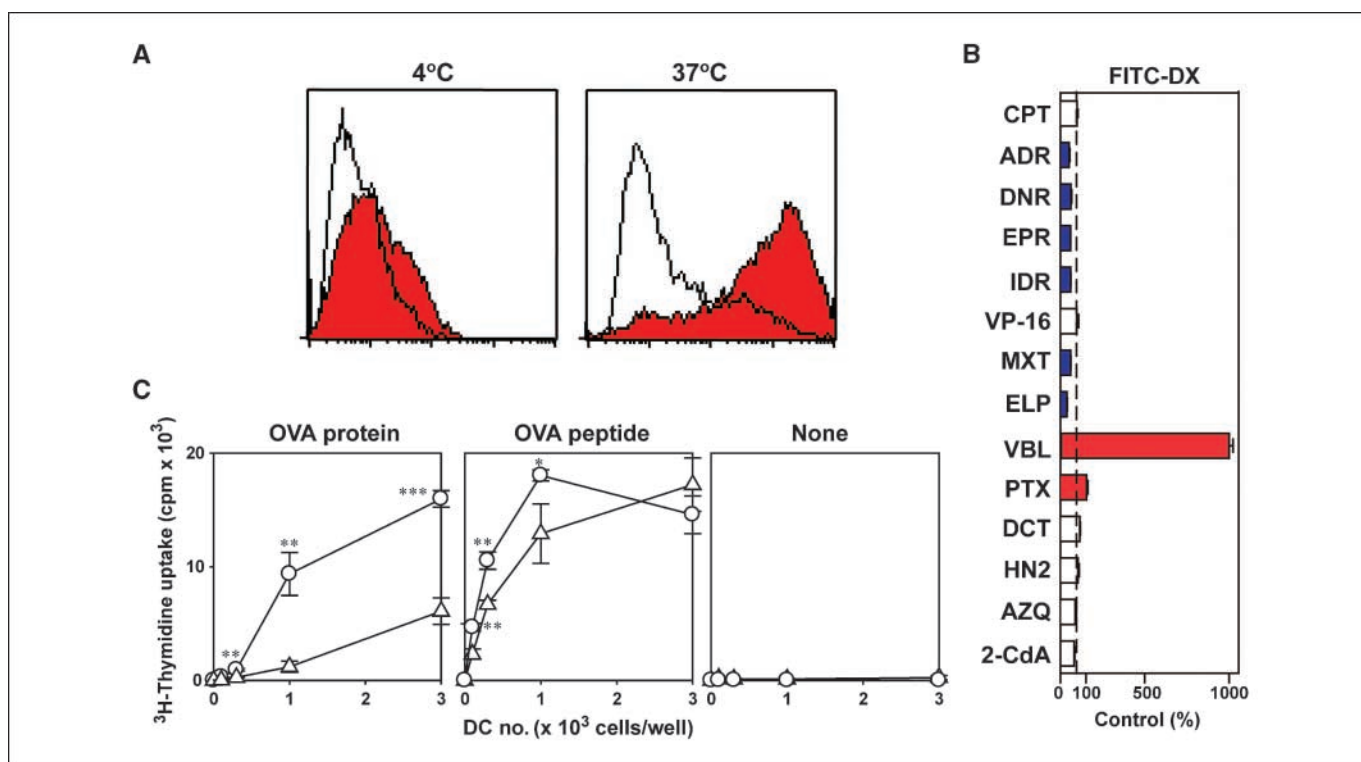


Figure 6. Vinblastine-induced augmentation of FITC-DX uptake and antigen cross-presentation by BM-DCs. **A**, BM-DCs were incubated for 24 h with 0.3 μ mol/L vinblastine (filled histograms) or vehicle alone (open), incubated with FITC-DX at 4°C or 37°C, and then examined for FITC fluorescent signals within the CD11c⁺ populations. **B**, following 24 h incubation with a test drug, BM-DCs were examined for FITC-DX uptake (mean fluorescence intensity) within the CD11c⁺ populations. Data shown are the means $\pm$ SD ($n = 3$) of relative activities (% of control) compared with the vehicle-treated control. Statistically significant ($P < 0.05$) and biologically relevant ($>30\%$) increase above or decrease is indicated in red or blue, respectively. **C**, BM-DCs were incubated for 24 h with 0.3 μ mol/L vinblastine (circles) or vehicle alone (triangles). The DC preparations were pulsed for 1 h with intact OVA protein (0.3 mg/mL), OVA₂₅₇₋₂₆₄ peptide (10 μ g/mL), or PBS alone, washed extensively, and added to the indicated cell numbers to microcultures of CD8 T cells (5×10^4 cells/well) purified from the OT-I transgenic mice. Data shown are the means $\pm$ SD ($n = 3$) of ³H-thymidine uptake. Statistically significant differences compared with the vehicle-treated control are indicated with asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

ligands (23). Thus, we measured FITC-DX uptake as an additional functional variable. Control BM-DCs treated with vehicle alone showed modest FITC-DX uptake at 37°C and minimal surface binding at 4°C. By marked contrast, DCs treated with vinblastine showed an augmented endocytic capacity (Fig. 6A). Quantitative analysis revealed 10-fold improvement of FITC-DX uptake after vinblastine treatment (Fig. 6B). Importantly, none of the other tested type 1 drugs exhibited such striking effects, except for paclitaxel augmenting FITC-DX incorporation modestly. These observations implied that vinblastine might promote the cross-presentation of exogenous antigens to CD8 T cells. In fact, vinblastine-treated BM-DCs were far more efficient than vehicle-treated DCs in presenting OVA protein to CD8 T cells purified from the OT-I T-cell receptor transgenic mice, in which a majority of CD8 T cells recognize the OVA₂₅₇₋₂₆₄ peptide presented in H2-K^b (Fig. 6C). Vinblastine treatment also augmented, albeit modestly, their ability to present OVA₂₅₇₋₂₆₄ peptide to the same OT-I CD8 T cells.

Although beyond the scope of the present study, we tested the effects of vinblastine on human DCs using commercially available DC preparations derived from CD34⁺ progenitors in the cord blood. Upon exposure to vinblastine at 0.3 μ mol/L, human DCs (as defined by CD1a expression in those cultures) increased the surface expression of MHC II, CD40, CD80, and CD86, elaborated IL-6, IL-8, RANTES, and MIP-1 α , and exhibited robust uptake of FITC-DX by

human DCs after vinblastine treatment (Supplementary Fig. S1). These *in vitro* observations showed the potential of vinblastine to trigger phenotypic and functional maturation of human DCs as well.

Discussion

Our unbiased functional screen of 54 chemotherapeutic agents has unveiled a striking diversity in their pharmacologic effects on maturation, survival, and growth of DCs. Based on the concentrations required to affect the three functional variables, we categorized the drugs into four classes. To our knowledge, this is the first published study in which diverse anticancer drugs are compared in parallel for their effects on DCs. Considering the important roles played by DCs in initiating and regulating host immune responses to cancer (1, 7), our screening results provide essential information that will allow a rational selection of chemotherapeutic agents for ultimate clinical outcome.

Suppression of host immunity is one of the major adverse effects of chemotherapy. Contrary to this general notion, 15 anticancer drugs were categorized as type 1 in our DC biosensor screening. These drugs were subsequently confirmed to induce at least one of the characteristic maturational changes in BM-DCs. Interestingly, most of the tested topoisomerase inhibitors (9 of 12) and antimicrotubule agents (3 of 5) were categorized as type 1, whereas

the remaining drugs in these two families were categorized as type 2. By contrast, only a few tested alkylating agents (2 of 14), an antimetabolite, and none of the platinum agents or hormonal agents satisfied the criteria for type 1. These observations may suggest an intriguing correlation between the functional property of delivering DC maturation signals and the pharmacologic mechanisms of action among the tested chemotherapeutic agents.

Vinblastine was regarded as the most prominent inducer of DC maturation among the tested type 1 drugs; it increased CD40, CD80, CD86, and MHC II expression, triggered IL-1 β , IL-6, and IL-12 p40 production, and augmented the capacity to activate allogeneic T cells. Interestingly, vinblastine seems to induce DC maturation both directly and indirectly. Vinblastine markedly improved the abilities of BM-DCs to incorporate FITC-DX and to cross-present OVA protein to CD8 T cells. Our findings may first seem contradictory to the general notion of vinblastine as a chemotherapeutic drug with immunosuppressive potentials. Indeed, vinblastine has been reported to inhibit mitogen-induced lymphocyte activation (24), induction of cytotoxic T-cell activity (25), tumoricidal potential of macrophages (26), natural killer cell-mediated cytotoxicity (27), antibody-dependent cell-mediated cytotoxicity (28), humoral immune responses (29), cellular immune protection against microbial infection (30), and host antitumor immunity (31). However, most of those immunosuppressive properties were observed for vinblastine at relatively high concentrations and/or after repeated administrations. Our data showed that vinblastine, at relatively low concentrations (0.1–1 μ mol/L), induced the phenotypic and functional maturation of mouse and human DCs. We recently observed that local injection of a small amount of vinblastine is sufficient to trigger *in situ* maturation of skin-resident DCs and boost humoral and cellular immune responses to a model antigen in mice. When injected at small amounts directly into B16 melanoma, vinblastine elicited a marked CTL activity against melanoma targets and interrupted otherwise progressive growth of B16 melanoma (32). Thus, we suggest that vinblastine produces opposing immunologic outcomes depending on administration doses. To the best of our knowledge, this is the first report documenting the ability of vinblastine to trigger the maturation of DCs.

Our findings with vinblastine are consistent with our previous results—two microtubule inhibitors, colchicine and podophyllotoxin, were identified as “hits” by DC biosensor-based screening of 880 Food and Drug Administration–approved drugs (15). With regard to underlying mechanisms, colchicine and podophyllotoxin both activated the nuclear factor κ B pathway in DCs. As observed with vinblastine, they both induced the production of IL-1 β , IL-6, and IL-12, but not TNF α . Interestingly, a synthetic microtubule inhibitor, CC-5079, was reported to suppress TNF α production by inhibiting phosphodiesterase type 4, an essential cyclic AMP–metabolizing enzyme known to be involved in lipopolysaccharide-activated TNF α responses (33). Vinblastine is one of the classic *Vinca* alkaloids that have greatly contributed to the clinical success of chemotherapy over the last four decades (34). At high concentrations, these agents directly interfere with proper spindle microtubule formation, thereby blocking cell mitosis and eventually leading to apoptosis. The *Vinca* alkaloids also stabilize microtubule dynamics at low concentrations, thereby affecting various cellular activities. Thus, we suggest that partial and temporal disruption of intracellular microtubule networks may be sensed by DCs as intrinsic danger signals.

In summary, we have screened a variety of chemotherapeutic agents in the DC biosensor system, categorized them based on their differential effects on three functional variables of DCs, and identified several structurally unrelated drugs capable of triggering DC maturation. Because DCs play crucial roles in regulating host immunity against tumor, our screening results will provide essential data sets for rational selection and combination of anticancer drugs for the treatment of patients with cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Dhodapkar MV, Dhodapkar KM, Palucka AK. Interactions of tumor cells with dendritic cells: balancing immunity and tolerance. *Cell Death Differ* 2008;15:39–50.
- Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoeediting. *Annu Rev Immunol* 2004;22:329–60.
- Kim R, Emi M, Tanabe K, Arihiro K. Tumor-driven evolution of immunosuppressive networks during malignant progression. *Cancer Res* 2006;66:5527–36.
- Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005;5:263–74.
- O'Neill DW, Adams S, Bhardwaj N. Manipulating dendritic cell biology for the active immunotherapy of cancer. *Blood* 2004;104:2235–46.
- Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol* 2005;5:296–306.
- Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007;449:419–26.
- Lake RA, Robinson BW. Immunotherapy and chemotherapy—a practical partnership. *Nat Rev Cancer* 2005;5:397–405.
- Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol* 2008;8:59–73.
- Casares N, Pequignot MO, Tesniere A, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005;202:1691–701.
- Spisek R, Charalambous A, Mazumder A, Vesole DH, Jagannath S, Dhodapkar MV. Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. *Blood* 2007;109:4839–45.
- Proietti E, Greco G, Garrone B, et al. Importance of cyclophosphamide-induced bystander effect on T cells for a successful tumor eradication in response to adoptive immunotherapy in mice. *J Clin Invest* 1998;101:429–41.
- Ghiringhelli F, Larmonier N, Schmitt E, et al. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol* 2004;34:336–44.
- Mullins DW, Burger CJ, Elgert KD. Paclitaxel enhances macrophage IL-12 production in tumor-bearing hosts through nitric oxide. *J Immunol* 1999;162:6811–8.
- Mizumoto N, Gao J, Matsushima H, Ogawa Y, Tanaka H, Takashima A. Discovery of novel immunostimulants by dendritic cell-based functional screening. *Blood* 2005;106:3082–9.
- Matsue H, Matsue K, Walters M, Okumura K, Yagita H, Takashima A. Induction of antigen-specific immunosuppression by CD95L cDNA-transfected “killer” dendritic cells. *Nat Med* 1999;5:930–7.
- Chu E, Takimoto CH, McLeod HL, et al. Pharmacology of cancer chemotherapy. In: DeVita VT, Jr., Hellman S, Rosenberg SA, editors. *Cancer: Principles and practice of oncology*. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 307–422.
- Matsue H, Yang C, Matsue K, Edelbaum D, Mummert ME, Takashima A. Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and

- dexamethasone) on bidirectional dendritic cell-T cell interaction during antigen presentation. *J Immunol* 2002; 169:3555–64.
19. Mizumoto N, Kumamoto T, Robson SC, et al. CD39 is the dominant Langerhans cell-associated ecto-ATPase: modulatory roles in inflammation and immune responsiveness. *Nat Med* 2002;8:358–65.
 20. Boes M, Cerny J, Massol R, et al. T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* 2002;418:983–8.
 21. Mizumoto N, Tanaka H, Matsushima H, Vishwanath M, Takashima A. Colchicine promotes antigen cross-presentation by murine dendritic cells. *J Invest Dermatol* 2007;127:1543–6.
 22. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 1995;182:389–400.
 23. Bot FJ, Schipper P, Broeders L, Delwel R, Kaushansky K, Lowenberg B. Interleukin-1 α also induces granulocyte-macrophage colony-stimulating factor in immature normal bone marrow cells. *Blood* 1990;76:307–11.
 24. Greene WC, Parker CM, Parker CW. Colchicine-sensitive structures and lymphocyte activation. *J Immunol* 1976;117:1015–22.
 25. Dye ES, North RJ. Adoptive immunization against an established tumor with cytolytic versus memory T cells. Immediate versus delayed onset of regression. *Transplantation* 1984;37:600–5.
 26. Sharma SD, Piessens WF. Tumor cell killing by macrophages activated *in vitro* with lymphocyte mediators. III. Inhibition by cytochalasins, colchicine, and vinblastine. *Cell Immunol* 1978;38:276–85.
 27. Katz P, Zaytoun AM, Lee JH, Jr. Mechanisms of human cell-mediated cytotoxicity. III. Dependence of natural killing on microtubule and microfilament integrity. *J Immunol* 1982;129:2816–25.
 28. Strom TB, Garovoy MR, Carpenter DB, Merrill JP. Microtubule function in immune and nonimmune lymphocyte-mediated cytotoxicity. *Science* 1973;181:171–3.
 29. Moav N, Steinberg L, Frensdorff A. Effect of vinblastine on the early events in the humoral immune response of mice to SRBC. *Int Arch Allergy Appl Immunol* 1976;52:257–65.
 30. McGregor DD, Logie PS. The mediator of cellular immunity. VI. Effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection. *J Exp Med* 1973;137:660–74.
 31. Klenner P, Bubenik J, Jandeksek J. Influence of vinblastine on antitumor immunity in mice. *Neoplasma* 1973;20:239–42.
 32. Tanaka H, Matsushima H, Nishibu A, Clausen BE, Takashima A. Dual therapeutic efficacy of vinblastine as a unique chemotherapeutic agent capable of inducing dendritic cell maturation. *Cancer Res* 2009; 69:6987–94.
 33. Zhang LH, Wu L, Raymon HK, et al. The synthetic compound CC-5079 is a potent inhibitor of tubulin polymerization and tumor necrosis factor- α production with antitumor activity. *Cancer Res* 2006;66:951–9.
 34. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* 2004;4:253–65.

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