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Tumor-induced macrophages (Mφs) mediate immunosuppression, in part, through increased production of factors that suppress T cell responsiveness and underproduction of positive regulatory cytokines. Pretreatment of tumor-bearing host (TBH) Mφs with the anticancer agent paclitaxel (Taxol) partially reverses tumor-induced Mφ suppressor activity, suggesting that paclitaxel may restore TBH Mφ production of proimmune factors. Because paclitaxel demonstrates LPS-mimetic capabilities and increased production of the LPS-induced immunostimulatory cytokine IL-12 could account for enhanced T cell responsiveness, we investigated whether paclitaxel induces Mφ IL-12 production. Tumor growth significantly down-regulated Mφ IL-12 p70 production through selective dysregulation of IL-12 p40 expression. LPS stimulation failed to overcome tumor-induced dysregulation of p40 expression. In contrast, paclitaxel significantly enhanced both normal host and TBH Mφ IL-12 p70 production in vitro, although TBH Mφ IL-12 production was lower than that of similarly treated normal host Mφs. Paclitaxel enhanced p40 expression in a dose-dependent manner. Through reconstituted Mφ IL-12 expression, paclitaxel pretreatment relieved tumor-induced Mφ suppression of T cell alloreactivity. Blocking Mφ NO suppressed paclitaxel’s ability to induce IL-12 production. This suggests that paclitaxel-induced activities may involve a NO-mediated autocrine induction pathway. Collectively, these data demonstrate that paclitaxel restores IL-12 production in the TBH and ascribe a novel immunotherapeutic component to the pleiotropic activities of NO. Through its capacity to induce IL-12 production, paclitaxel may contribute to the correction of tumor-induced immune dysfunction. 


Macrophages (Mφ) serve vital roles in host defense against tumors, including tumor cytotoxicity and stimulation of antitumor lymphocytes. However, tumors circumvent these host-mediated immune activities through the production and release of immunomodulatory factors, such as IL-10, PGE₂, and TGF-β, that adversely alter Mφ function and phenotype. These tumor-derived factors generate immunoregulatory Mφs that inhibit T cell responses and that are tumoricidally dysfunctional (reviewed in Ref. 1). Identification of the molecular and cellular origins of tumor-induced changes in Mφ function will increase opportunities for immunotherapeutic intervention. Recent studies (2–4) ascribed immune-activating properties to paclitaxel (Taxol), suggesting that chemotherapeutic applications of paclitaxel may impart novel immunotherapeutic activities on tumor-induced Mφs.

A plant-derived diterpenoid (5), paclitaxel first gained recognition as a potent inhibitor of cell cycle progression, leading to neo-plastic cell death and arrest of tumor progression (6–8). Paclitaxel demonstrated significant antitumor efficacy in human clinical trials (9) and became the chemotherapeutic agent of choice for first-line treatment of metastatic breast cancers (10). Paclitaxel’s primary mechanism of antineoplastic activity rests in its ability to irreversibly polymerize αβ tubulin, thereby disrupting cellular microtubule networks (11). Additionally, paclitaxel enhances tumor cell radiosensitivity (12) and sensitivity to immune effectors such as TNF-α (13).

In addition to its well-characterized chemotherapeutic activities, paclitaxel has profound cell cycle-independent effects on murine Mφs (2–4, 14). Paclitaxel induces normal host (NH) Mφ responses similar to those generated by bacterial LPS (15, 16), including enhanced NO (2, 17), TNF-α (18), IL-1β (19), and superoxide anion (20) production and induction of NF-κB expression (21). Through increased TNF-α and NO production, paclitaxel enhances in vitro tumor cell cytotoxicity (17).

Paclitaxel’s most significant immunotherapeutic activity, from a therapeutic standpoint, may be its capacity to reverse tumor-induced Mφ-mediated immune suppressor activities. Paclitaxel pretreatment of tumor-bearing host (TBH)-derived Mφs modulated suppression of alloantigen-activated T cell responsiveness (3) and concurrently enhanced Mφ antitumor cytotoxicity in vitro (3). Paradoxically, both the cytotoxic (3, 17) and immunosuppressive (22) functions of TBH Mφs are mediated partially through the overproduction of reactive nitrogen and oxygen intermediates, including NO, and proinflammatory cytokines, such as TNF-α. However, ablation of Mφ-derived suppressor molecules NO and TNF-α failed to fully overcome the suppressive effects induced by tumor growth in our model system (D. W. Mullins and K. D. Elgert, unpublished observation), leading to speculation that TBH Mφs may be deficient in immunostimulatory factor production. The capacity of the Mφ-derived cytokine IL-12 to promote cell-mediated antitumor immune responses during tumor growth (23, 24) and the LPS-mimetic activities of paclitaxel (15, 16, 25–28) suggested to us that paclitaxel may reverse tumor-induced suppressor activities....
through reconstitution of Mδ IL-12 production in the TBH. We report that fibrosarcoma growth dysregulates Mδ production of IL-12, compromising antitumor activity, and that paclitaxel induces IL-12 expression through a NO-dependent autocrine induction mechanism. Paclitaxel-activated Mδs coexpress cytotoxic and immunostimulatory activities that collectively may impart significant antitumor immunotherapeutic functions.

Materials and Methods

Murine tumor model

Eight- to 12-wk-old BALB/c (H-2b) male mice (Harlan Sprague Dawley, Madison, WI) were used as source for normal host and TBH lymphocytes. A BALB/c nonmetastatic methylcholanthrene-induced transplantable fibrosarcoma, designated Meth-KDE, was used as described (29). The use of a nonmetastatic tumor facilitates the study of tumor-induced distal Mδ populations. The Meth-KDE fibrosarcoma induces significant systemic immunosuppression through the production of the soluble suppressor cytokines IL-10, TGF-β1, and PGE2 (30). Tumors were induced by i.m. injection of 4 x 103 transplanted Meth-KDE cells, and palpable tumors developed within 10 days. TBH BALB/c mice were used 21 days after tumor inoculation; tumor-bearing Mδ suppressor activity was maximal at this time (31), without cachexia or necrosis. Eight- to 12-wk-old male C3H-HeJ (H-2k) mice were the source of alloantigen stimulator cells. All comparisons were tested for significance by Student’s test, and all comparisons are significant at the p < 0.05 level, unless otherwise stated.

IL-12 protein measurement

Either NH or TBH Mδs (4 x 105 cells/well) were cultured with the indicated reagents in 96-well flat-bottom plates. Supernatants were collected and immediately assayed for IL-12 using a p70-specific ELISA (IL-12 DuoSet; R&D Systems, Minneapolis, MN) per the manufacturer’s protocol. The ELISA consisted of anti-mouse IL-12 p70 capture Ab adhered to high affinity protein binding plates (Nunc (Naperville, IL) MaxiSorp ELISA plates), biotinylated secondary Ab, and HRP-conjugated streptavidin detection reagent. ELISAs were developed with Sigma tetramethylbenzidine liquid substrate system, and absorbance was determined at 450 nm using an MR-600 microplate reader (Dynex, Chantilly, VA). The limit of detection in our hands was approximately 10 pg/ml.

Ribonuclease protection assay analysis of IL-12 subunit expression

Either NH or TBH Mδs (5.0 x 105 cells) were cultured in serum-free medium with the indicated reagents. Mδs were cultured for 2 h in 24-well flat-bottom plates (Corning Cell Wells, Corning, NY), collected, and stored in liquid nitrogen for subsequent analysis. Total mRNA was extracted using the RNeasy total RNA system following cell disruption by passage through a Qiashredder (both from Qiagen, Chatsworth, CA), per the manufacturer’s protocol.

Antiserine riboprobes were constructed from specific IL-12 cDNA templates (PharMingen) using the MAXScript T-7 in vitro transcription system (Ambion, Austin, TX) and labeled using the BrightStar psoralen-biotin method (Ambion). Ribonuclease protection assay (RPA) was performed using the Ambion RPA-II system, per the manufacturer’s protocol. RPA products were separated on a 5% polyacrylamide gel containing 8 M urea, transferred to nylon membrane, and visualized with the Ambion BioDetect nonisotopic detection system on Kodak (Rochester, NY) BioMax MR-1 autoradiography film. Images were digitized, and densitometric analyses were performed using NIH-Image software for Macintosh.

T cell alloreactivité assays

Either NH or TBH Mδs (2 x 105 cells) were added to NH T cell cultures (2 x 105 cells) in 96-well U-bottom tissue culture plates (Nunc). Some Mδs were pretreated with paclitaxel or LPS for 4 h before addition to T cell cultures. Irradiated (2000 rad) whole splenic cell preparations (4 x 105 cells) from C3H (H-2k) mice were used as allogeneic stimulator cells. Neutralizing anti-IL-12 mAb (C17.8, 10 μg/ml) or rat IgG2a isotype control mAb (10 μg/ml) was added to some cultures. Eighteen hours before harvest, cultures were pulsed with 1 μCi per well [3H]Tdr, (sp. act. 6.7 Ci/mM, DuPont-NEN Research Products, Boston, MA). Cells were harvested with a Skatron (Chantilly, VA) automated cell harvester and sample activities determined using a Beckman Coulter (Fullerton, CA) LS 6000SC scintillation counter.

Macrophage nitrite production

Following incubation, supernatants (100 μl) were collected from all antigen-activated T cell proliferation cultures. NO was analyzed by measuring total nitrite levels in culture supernatants using the Griess reagent, as described elsewhere (34). Briefly, 100 μl of Mδ supernatants was added to 100 μl Griess reagent (0.1% naphthylenediamine dihydrochloride, 1.0% sulfanilamide, 2.5% H3PO4, Sigma) and incubated at room temperature for 10 min; absorbance was read at 570 nm. A sodium nitrite (Sigma) standard curve was used to calculate nitrite content in supernatants. Nitrite was not detected in RPMI 1640 medium alone.

Statistics and calculations

Cells from 6 to 10 NH or TBH mice were pooled for each experiment. Triplicate cultures were tested in specific ELISAs, Griess reagent tests, and proliferation assays. Data are presented as means ± SEM of triplicate independent determinations. All experiments were repeated at least three times; representative experiments are shown. All comparisons were tested for significance by Student’s t test, and all comparisons are significant at the p < 0.05 level, unless otherwise stated.

Results

Tumor growth dysregulates Mδ production of IL-12 p70 heterodimer

In the presence of tumor-induced Mδs, T cell proliferative responses are suppressed, suggesting that neoplastic tissues subvert Mδ function to favor tumor growth (1). In our model system,
FIGURE 1. Tumor growth dysregulates Mφ production of IL-12 p70 heterodimer. Either 4 × 10^5 NH or TBH peritoneal Mφs were cultured in 200 μl of serum-free medium with IFN-γ (10 U/ml). LPS (0.1–10.0 μg/ml) was added to some wells at the start of culture. After 24 h, supernatants were collected and assayed for IL-12 heterodimer by p70-specific ELISA. Data are averages and SEM of triplicate independent determinations from one of five similar experiments. Untriggered TBH Mφ IL-12 production was significantly (p < 0.05) lower than similarly activated NH Mφs, and LPS (0.1–10.0 μg/ml)-induced TBH Mφ IL-12 production was significantly (p < 0.005) less than similarly activated NH Mφs at all doses.

tumor-derived factors such as PGE₂, TGF-β1, and IL-10 induce systemic dysregulation of Mφ functions (30). Therefore, we determined whether tumor growth compromises IL-12 production by Mφs derived from tumor-distal compartments. NH or TBH peritoneal Mφs (4 × 10^5 cells) were IFN-γ primed (10 U/ml) and cultured without or with LPS (0.1–10.0 μg/ml) for 24 h. Supernatants were collected and assayed for IL-12 heterodimer by p70-specific ELISA (Fig. 1). IL-12 production in the absence of IFN-γ priming was minimal (not shown), consistent with the findings that the IL-12 p40 gene promoter is primed by IFN-γ (35). Tumor growth significantly (p < 0.05) inhibited IFN-γ-induced IL-12 production (74% decrease compared with NH Mφs). LPS triggering stimulated NH Mφ IL-12 production, leading to greater than a 120% increase in IL-12 at the optimal dose of LPS (1.0 μg/ml). LPS enhanced IFN-γ-primed TBH Mφ production of IL-12 by 115% at the optimal dose (1.0 μg/ml), as compared with untriggered TBH Mφs; however, the level of IL-12 production by TBH Mφs was significantly (p < 0.005) less than similarly treated NH Mφs at all doses of LPS tested. TBH Mφ IL-12 production, regardless of LPS triggering, never exceeded that of untriggered NH Mφs, demonstrating a serious lesion in the response of TBH Mφ populations. Splenic Mφ cultures produced similar profiles (not shown).

Tumor growth dysregulates Mφ IL-12 p40 expression

The bioactive IL-12 heterodimer is composed of the constitutively expressed p35 subunit and the inducible p40 subunit (36). Because the p70 heterodimer is necessary for biologic activity and p70 production is regulated by the level of available p40, we determined whether tumor growth dysregulates Mφ p40 expression in our murine fibrosarcoma model. Either NH or TBH peritoneal Mφs (5 × 10^5 cells) were IFN-γ primed (10 U/ml) and cultured for 4 h (optimal time) without or with LPS (1.0 μg/ml, optimal dose). Total RNA was collected and p40 expression was measured by RPA (Fig. 2A). LPS failed to enhance p40 expression in tumor-induced Mφs, further defining the lesion in TBH Mφ function.

FIGURE 2. Tumor growth dysregulates Mφ IL-12 p40 expression. Either 5 × 10^6 NH or TBH peritoneal Mφs were cultured as described for 4 h, and total RNA was collected. LPS (1.0 μg/ml) was added to some wells at the start of culture. A, RPA analysis of IL-12 p40 expression using p40-specific primers, with relative densitometric units as determined using NIH-Image. B, Expression of the housekeeping gene GAPDH, with relative densitometric units. Data are representative results from one of three similar experiments.

LPS did induce p40 expression in NH cells, consistent with the reports of others (37), suggesting that tumor growth dysregulates IL-12 heterodimer production through abrogated activation response of the inducible p40 subunit. Expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was consistent throughout all samples (Fig. 2B).

Paclitaxel reconstitutes Mφ IL-12 production

Paclitaxel has Mφ-activating functions in vitro (2, 17) and in vivo (3), and paclitaxel pretreatment of TBH Mφs reverses tumor-induced Mφ suppression of T cell proliferation (3). Because administration of exogenous IL-12 can promote antitumor immune function and paclitaxel can activate expression of several LPS-inducible genes, we hypothesized that reconstituted Mφ IL-12 production may account for restored T cell proliferative responses. Therefore, we determined whether paclitaxel could induce Mφ IL-12 production. NH or TBH (4 × 10^5) peritoneal Mφs were primed with IFN-γ (10 U/ml) and cultured without or with paclitaxel (0.1–10 μM) for 24 h. Supernatants were collected and assayed for IL-12 heterodimer, as described. Paclitaxel (10 μM) significantly (p < 0.05) enhanced both NH and TBH Mφ IL-12 production (Fig. 3), increasing IL-12 levels by 64% and 112%, respectively. Paclitaxel doses as low as 0.1 μM enhanced NH Mφ IL-12 production, although doses more than 10 μM did not further enhance NH or TBH Mφ IL-12 production (not shown). Splenic Mφ cultures produced similar profiles, although total IL-12 production by all samples was approximately 50% less than comparably treated peritoneal Mφ cultures (not shown). The induction of
IL-12 was not an artifact of endotoxin contamination because boiling paclitaxel for 60 sec abrogated its capacity to induce IL-12 (not shown).

**Paclitaxel overcomes tumor-induced suppression of Mφ IL-12 p40 expression**

Abrogation of TBH Mφ p40 expression (see Fig. 2A) and paclitaxel-mediated reconstitution of TBH Mφ IL-12 heterodimer production (see Fig. 3) suggest that paclitaxel may induce IL-12 p40 expression. To test this hypothesis, NH or TBH peritoneal Mφs (5 × 10^6 cells) were IFN-γ primed and cultured for 4 h without or with various doses of paclitaxel (0.1–10 μM); p40 expression was assessed by RPA (Fig. 4). Paclitaxel induced IL-12 p40 expression in a dose-dependent manner in both NH (Fig. 4A) and TBH Mφs (Fig. 4B). Paclitaxel doses more than 10 μM did not further enhance p40 expression (not shown). Expression of GAPDH was consistent throughout all samples.

**Paclitaxel induces Mφ IL-12 production through NO**

We (2, 3) and others (17) have shown that paclitaxel stimulates Mφ production of the pleiotropic effector molecule NO. Because NO regulates IL-12 gene expression in the RAW264.7 Mφ cell line (38), we determined whether paclitaxel-induced NO may be involved in the stimulation of murine Mφ IL-12 production (Fig. 5). IFN-γ-primed (10 U/ml) NH or TBH peritoneal Mφs were cultured for 24 h without or with paclitaxel (10 μM) and the specific NO-inhibitor NMMA (0.1 mM, sufficient to eliminate measurable NO production without compromising Mφ viability). Paclitaxel significantly (p < 0.05) enhanced NH Mφ IL-12 production, but paclitaxel’s capacity to induce IL-12 was blocked with the addition of NMMA. Tumor growth negatively modulates but does not abrogate paclitaxel-induced Mφ NO production (3, 4). Paclitaxel significantly (p < 0.05) enhanced TBH Mφ IL-12 production, and paclitaxel-mediated IL-12 production was NO-dependent (Fig. 5). In the absence of NO production, paclitaxel-induced NH or TBH Mφ IL-12 production was not significantly different from basal levels, and NMMA alone did not influence IL-12 production (Fig. 5).

**Paclitaxel-induced IL-12 reverses tumor-induced suppression of T cell alloreactivity**

Tumor growth induces Mφ suppressor activities (1), compromising T cell proliferative responses in alloantigen-activated MLR.
Much research has focused on defining how tumors induce Mφ-suppressor functions (3). Because paclitaxel induces Mφ IL-12 production and IL-12 can enhance T cell proliferative responses (42–44), we assessed whether paclitaxel-induced IL-12 was responsible for the reversal of TBH Mφ-mediated immune suppression by measuring lymphocyte responsiveness in culture with paclitaxel-pretreated Mφs in the absence or presence of IL-12. NH BALB/c (H-2<sup>b</sup>) CD4<sup>+</sup> T cell (2 × 10<sup>5</sup> cells) responsiveness to alloantigen stimulation (4 × 10<sup>5</sup> X-irradiated C3H [H-2<sup>k</sup>] splenocytes) was assessed in MLR with TBH Mφs (2 × 10<sup>5</sup> cells). Mφs were pretreated (4 h, optimal time) without or with paclitaxel (10 μM) before addition to the MLR. Alloreactivity was assessed in the absence or presence of neutralizing anti-IL-12 mAb (C17.8, 10 μg/ml), anti-IL-2 (S4B6), or rat IgG2a isotype control mAb (10 μg/ml) was added to some cultures. Eighteen hours before harvest, cells were pulsed with 1 μCi of [3H]TdR, and proliferation was determined. Data are magnitude of response relative to alloantigen-activated T cell proliferation in the presence of untreated NH Mφs (control cpm was 86,460). Proliferation of NH responder cells or X-irradiated stimulator cells alone was less than 5% of control. Data are averages and SEM of triplicate independent determinations from one of three similar experiments. *, p < 0.05, compared with control.

Because Mφ-derived NO plays a role in the reversal of tumor-induced Mφ suppressor functions, CD4<sup>+</sup> T cell alloantigen reactivity was assessed, as described, in the absence or presence of the NO inhibitor NMMA (Fig. 7). NO production, as measured by the Griess reaction, was 10.1 ± 0.3 μM and 37.4 ± 2.8 μM in cultures containing untreated or paclitaxel-treated TBH Mφs, respectively. NO production in cultures containing NMMA was beyond the limit of detection of our assay system. Paclitaxel pretreatment failed to relieve tumor-induced Mφ suppressor activity in the presence of the NO inhibitor, demonstrating that NO is involved in the paclitaxel-mediated reversal of tumor-induced Mφ suppressor activity. Addition of exogenous IL-12 (1000 pg/ml, approximately equal to paclitaxel-induced [10 μM] IL-12 production by TBH Mφs; see Fig. 3) to NMMA-containing cultures reconstituted T cell proliferative response (Fig. 7). Data are expressed as magnitude of response relative to proliferation in the presence of unactivated NH Mφs (control cpm was 80,710; not shown).

**Discussion**

Tumor growth significantly alters Mφ phenotype and function. Much research has focused on defining how tumors induce Mφ production of immunomodulatory signals (reviewed in Ref. 1), demonstrating that these factors are important mediators of immunosuppression during cancer. However, our recent studies on the immune activities of the anticancer drug paclitaxel led us to address tumor-induced immune suppression from a different point of view. Perhaps, in addition to overexpression of inhibitory cytokines, host antitumor response may be compromised by the lack of a necessary priming, activating, or costimulatory signal(s), preventing the induction of immune responses to tumors. An ideal
candidate for study was Mφ-derived IL-12 (reviewed in Ref. 45), a novel heterodimeric cytokine that enhances antitumor cytotoxic responses (46, 47), and a single factor that possesses the potential to correct many TBH immune dysfunctions.

Pleiotropic in its activities, IL-12 induces T cell (48) and NK cell (49, 50) IFN-γ production and promotes T cell development along the Th1 pathway (51–54). These functions led to speculation that IL-12 could be useful as an anticancer immunotherapeutic agent (55, 56), and clinical trials using direct administration (23, 57) and gene therapy approaches (24, 58, 59) have yielded promising results. The capacity of exogenous IL-12 to partially restore TBH immunocompetence suggests that tumor-induced Mφ dysfunction may involve decreased production of IL-12. Dysregulation of Mφ IL-12 may occur among both tumor-proximal and -distal immune cell populations if the tumor produces or induces the production of immunomodulatory factors such as IL-10 and TGF-β1 (60), as occurs in our tumor model (1, 30). Handel-Fernandez et al. (61) reported that a nonmetastatic mammary adenocarcinoma (D1-DMBA-3) dysregulated peritoneal exudate Mφ p40 expression through PGE2 and phosphatidyl serine. Using a p70-specific ELISA to avoid the potential interference of excess free p40 or p40 homodimers, we found that murine fibrosarcoma growth significantly (p < 0.05) inhibited IFN-γ-induced IL-12 p70 production by 74%. Although LPS enhanced IFN-γ-primed TBH Mφ IL-12, the level of production remained significantly (p < 0.005) less than similarly treated NH Mφs (Fig. 1). Most striking, optimal LPS triggering of TBH Mφs could not induce IL-12 production even to the levels of unprimed IFN-γ-primed NH Mφs. Tumor-induced dysregulation of IL-12 occurs at the level of transcription, since p40 expression was largely abrogated in tumor-induced Mφs regardless of priming or triggering with IFN-γ and LPS, respectively (Fig. 2). Because IL-12 drives the induction of the Th1-type immune response (the most effective antitumor response), the dysregulation of IL-12 production could effectively disable this arm of the immune response and allow tumors to grow unhindered.

Recognizing that tumor growth negatively regulates Mφ production of immunostimulatory factors, it followed that therapeutic agents that reconstitute immune activity through indirect action could be inducing the production of stimulatory cytokines. Pretreatment of TBH Mφs with the anticancer chemotherapeutic agent paclitaxel partially reversed Mφ-mediated suppressor activity (3). This suggested that paclitaxel, which has demonstrated Mφ-activating functions in vitro (2, 17) and in vivo (3), may enhance T cell reactivity by activating Mφ IL-12 production. Analyzing for IL-12 p70 showed that paclitaxel significantly (p < 0.05) enhanced IFN-γ-primed Mφ IL-12 production regardless of tumor growth (Fig. 3), and RPA analysis showed that paclitaxel activates p40 expression in a dose-dependent manner (Fig. 4).

Although our data strongly suggest that paclitaxel induces IL-12 production and p40 expression, the mechanisms remained to be clarified. One possibility involved paclitaxel-mediated induction of Mφ NO production. NO regulates IL-12 gene expression in the RAW264.7 Mφ cell line (38), and paclitaxel stimulates Mφ production of NO (2, 3, 17). We determined that NO is an essential mediator of IL-12 production in our model system. Paclitaxel (10 μM) significantly (p < 0.05) enhanced both NH and TBH Mφ IL-12 production, but paclitaxel’s capacity to induce IL-12 was substantially blocked with the abrogation of NO production. These data suggest that a primary mechanism of IL-12 induction by paclitaxel may involve autocrine signaling through Mφ-derived NO.

Although tumors mediate functional and phenotypic changes in Mφ populations, T cells may not be functionally altered, opening the possibility that restoration of Mφ IL-12 production through chemotherapeutics may be beneficial to the TBH. Paclitaxel pretreatment of TBH Mφs partially reversed tumor-induced suppressor functions (3), and we determined that paclitaxel-mediated Mφ IL-12 production was responsible for the reversal of immune suppression (Fig. 6). Neutralization of IL-12 activity in T cell alloreactivity cultures abrogated paclitaxel-mediated reversal of tumor-induced suppression, suggesting that paclitaxel’s apparent immunotherapeutic activity is achieved through the induction of Mφ IL-12 production. This suggests that the TBH immune system may be fully capable of responding to cancer if provided with appropriate stimulatory signals.

Interestingly, paclitaxel-induced Mφ NO production plays a role in the restoration of immunocompetence. In the presence of the NO inhibitor NMMA, paclitaxel pretreatment failed to relieve tumor-induced Mφ suppressor activity (Fig. 7). These results provide an intriguing link between IL-12 and NO, molecules with disparate function in tumor immunity. In terms of signaling mechanisms, Schwacha and Eisenstein (62) reported that IL-12 was necessary for the induction of NO production in an infectious disease model, and Wigginton et al. (63) showed that IL-12 primed TBH Mφs for enhanced NO production. These studies suggest that IL-12, through its capacity to induce IFN-γ production, leads to priming of Mφ populations for enhanced NO production (IL-12 has no direct effect on Mφ NO production; D. W. Mullins and K. D. Elgart, unpublished observation). This report suggests an additional regulatory pathway by demonstrating that NO can induce Mφ IL-12 production through an autocrine mechanism, thus perpetuating the cycle of activation and enhancing the potential for strong cell-mediated immune responses.

In terms of T cell reactivity, reconstitution of alloantigen-activated T cell proliferative response in the presence of NO and loss of response in the absence of NO production may seem counterintuitive. NO is a potent antagonist of lymphocyte proliferative responses and can severely dysregulate T cell reactivity (64–66). In our model system, we observed that LPS enhances Mφ NO production, leading to drastic decreases in T cell alloreactivity, and blocking NO with specific inhibitor relieves suppression. In the TBH, Mφs are primed for enhanced NO production on LPS signaling, but these same cells are incapable of substantial IL-12 production (Fig. 1). When LPS-activated TBH Mφs are added to alloantigen-activated NH CD4+ T cells, proliferation remains severely compromised, partly because of high levels of Mφ NO. In contrast, paclitaxel pretreatment induces TBH Mφ production of NO at lower levels, which may have direct inhibitory effects of the T cells; however, that Mφ-derived NO also acts in an autocrine manner to induce Mφ IL-12 production, which in turn enhances T cell proliferative responses (Fig. 5). Administration of exogenous IL-12 enhances IFN-γ in TBH (67), which in turn can enhance Mφ-inducible NO synthase expression and NO production in situ, leading to an amplification of antitumor responses. These results suggest that the restoration of IL-12 has significant positive implications for the TBH.

Controversy surrounds human monocyte/Mφ production of NO (68), leading to questions about the relevancy of murine data to human oncology studies. Human Mφs do possess the gene and functional protein for NO production (69), and NO production occurs in vitro (69, 70). Recent data indicate that human peripheral blood mononuclear cells can produce moderate amounts of NO in vivo (70). From the opposing view, it could be argued that lack of NO production by human Mφs may contribute to tumor-induced immunosuppression by the loss of IL-12 and IFN-γ induction mechanisms. Regardless of human Mφ NO production, further
investigation of Mφ responsiveness to paclitaxel in human cancer patients is warranted.

Collectively, these results demonstrate that the antineoplastic agent paclitaxel restores IL-12 production in the TBH, a novel mechanism for the immunotherapeutic activity of paclitaxel. Further, these data ascribe a novel signaling component to the pleiotropic activities of NO. We suggest that the immune activities of paclitaxel should be considered in a clinical context, because recognition of paclitaxel’s immune-activating properties may lead to optimization of current chemotherapeutic treatment regimens.

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