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IL-10 Mediates Sigma$_1$ Receptor-Dependent Suppression of Antitumor Immunity[1]

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Sigma receptors are unique endoplasmic reticulum proteins that mediate signaling for a variety of drugs. We determined the effect of sigma$_1$ receptor agonists on immune responses in a syngeneic lung cancer model. Sigma$_1$ receptor agonists, including cocaine, up-regulated splenocyte IL-10 mRNA and protein production in vitro in a sigma receptor-dependent, pertussis toxin-sensitive manner. In vivo, sigma$_1$ receptor agonists promoted tumor growth and induced IL-10 at the tumor site. Increased tumor growth was prevented by administration of specific Abs to IL-10 or by administration of specific sigma$_1$ receptor antagonists. We report that sigma$_1$ receptor ligands, including cocaine, augment tumor growth through an IL-10 dependent mechanism. ***The Journal of Immunology,*** **2003, 170: 3585–3591.**

Sigma receptors, widely distributed in neuronal and non-neuronal tissues, are unique drug-binding proteins (1). These receptors interact with a variety of compounds including cocaine, and dextromethorphan and benzomorphans such as pentazocine (2, 3). At least two subtypes of sigma receptors, classified as sigma$_1$ and sigma$_2$, are distinguishable by physiologic function and pharmacologic response.

Sigma ligands have potent immunoregulatory properties including the induction of IL-10 (4) and the suppression of IFN-γ and GM-CSF (5). In murine studies, treatment with sigma ligands prevented both graft-vs-host disease and delayed-type hypersensitivity granuloma formation (5). These studies indicate that sigma receptor-dependent signaling plays a role in immune-mediated responses. Cocaine, a sigma$_1$ receptor ligand, is also known to modulate immune function in vivo and in vitro (6, 7). Because immunocompetent animal models of tumorigenicity and tumor progression can serve as sensitive indicators of immune dysfunction, in the present studies we evaluated the capacity for sigma ligands to limit antitumor immune responses. Based on previous reports suggesting sigma receptor-dependent cytokine modulation (4, 5), we speculated that sigma receptor ligands would impact host antitumor immunity. We report for the first time that sigma$_1$ receptor-dependent signaling suppresses antitumor immune reactivity by up-regulating IL-10, resulting in augmentation of tumor growth.

Materials and Methods

Reagents

PRE-084 (2-(4-morpholino)ethyl-1-phenylcyclohexane-1-carboxylate) and SKF 10047 (sigma$_1$ receptor agonists), 4-IBP (high-affinity agonist for sigma$_1$ and moderate-affinity agonist for sigma$_2$ receptors), and BD1047 (sigma$_1$ receptor antagonist) were purchased from TOCRIS Cookson (Ellisville, MO). Cocaine hydrochloride (5–20 mg/ml in saline) was obtained from the National Institute of Drug Abuse. Reagents for PGE_2 assays were obtained from Cayman Chemical (San Diego, CA). Recombinant cytokines IL-10, IFN-γ, and TGF-β, as well as the corresponding Abs for these cytokine ELISAs were purchased from BD PharMingen (San Diego, CA). Recombinant TGF-β used for in vitro assays was purchased from R&D Systems (Minneapolis, MN). Staphylococcal entertoxin B (SEB)[3] and pertussis toxin (PT) were purchased from Sigma-Aldrich (St. Louis, MO). IL-10 mAb (JES-2A5) and control mAb (GL-113 5E) were provided by K. Moore (DNAX, Palo Alto, CA).

Mice

Pathogen-free male BALB/c mice (H-2^d) (8–12 wk of age) were obtained from Harlan Sprague Dawley (Indianapolis, IN) or Simonsen Laboratories (Gilroy, CA). Mice were maintained in the West Los Angeles Healthcare Center Animal Research Facility. All studies were approved by the institutional animal review committee.

Cell culture

The murine line 1 alveolar cell carcinoma (L1C2, H-2^d) cell line was used to establish the in vivo tumor model. L1C2 is a well-characterized line that is weakly immunogenic and shows progressive tumor growth in vivo (8). The cells were cultured in complete medium consisting of RPMI (Irvine Scientific, Santa Ana, CA) supplemented with 10% FCS (Gemini Bio-Products, Calabasas, CA) and antibiotics (penicillin and streptomycin; Gemini Bio-Products) and kept at 37°C in a humidified atmosphere containing 5% CO_2.

Evaluation of tumorigenicity

To determine the effect of sigma$_1$ receptor ligands on tumorigenicity in vivo, mice were pretreated for 2 wk with i.p. injections of PRE-084 (20 mg/kg), cocaine (5 mg/kg), or diluent control (saline) five times per week. For experiments with the sigma$_1$ receptor antagonist, BD1047 was given 30 min in advance of the agonist (i.e., cocaine or PRE-084) administration. Fourteen days following the initiation of PRE-084, cocaine, or diluent injections, 10^5 L1C2 cells were implanted s.c. in the suprascapular area. To identify the immunoregulatory role of IL-10 following sigma$_1$ receptor agonist administration in vivo, Ab blocking studies were performed for 2 wk in BALB/c mice that were pretreated with PRE-084, cocaine, or diluent

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[3] Abbreviations used in this paper: SEB, staphylococcal entertoxin B; PT, pertussis toxin.

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control as previously described and then were inoculated with 1×10^5 L1C2 cells by s.c. injection in the right suprascapular region. One day before tumor inoculation, mice received anti-IL-10 mAb (5 mg/kg) or control Ab (5 mg/kg) by i.p. injections three times a week for the duration of the experiment. Following implantation of tumor cells, mice continued to receive PRE-084, cocaine, antagonist BD1047, or diluent injections five times per week. Tumor growth was assessed as previously described (8). Two bisecting diameters of each tumor were measured with calipers and the volume was calculated using the formula $0.4 \times ab^2$, where *a* represents the larger diameter and *b* the smaller diameter.

Assessment of cytokine concentrations by ELISA

Non-necrotic tumors were isolated from the mice in the various treatment groups previously described for cytokine evaluation. IL-10, TGF-β, and IFN-γ levels were measured in the supernatant of tumor homogenates by cytokine-specific ELISA as previously described (9). To evaluate the sigma receptor-mediated production of cytokines independent of the influence of the tumor-bearing state, we assessed splenocyte cytokine production following administration of PRE-084 or cocaine in non-tumor-bearing mice. Mice received injections of the sigma receptor ligands 5 days per week for 2 wk. IL-10, TGF-β, and IFN-γ levels were measured in cultured splenocytes by cytokine-specific ELISA. Non-tumor-bearing BALB/c mice were pretreated with sigma receptor agonists and antagonists as previously described, and SEB (50 μg/mouse) was administered via a lateral tail vein injection. Two hours following SEB injection, blood samples were obtained by retro-orbital puncture, and IL-10 in the sera quantified by ELISA. To determine the specificity of $sigma_1$ receptor agonist-induced lymphocyte IL-10 production, BALB/c splenocytes were cultured with IL-2 (100 U/ml) for 3 days and then stimulated with agonists PRE-084 (0.3 μM), SKF 10047 (0.3 μM), or cocaine (0.3 μM) plus BD1047 (0.3 μM) for 24 h. To determine whether the $sigma_1$ agonist-induced IL-10 production could be inhibited by PT, splenocytes were cultured as previously described and PT (10 ng/ml) was added 24 h before treatment with the agonists. Following an additional 24 h, IL-10 secreted in the culture supernatants was quantified by ELISA.

PGE_2 determinations by enzyme immunoassay

All reagents for the PGE_2 assay were obtained from Cayman Chemical (Ann Arbor, MI). PGE_2 ELISA was performed according to the manufacturer's instructions as routinely performed in our laboratory (10). The plate was read at 405 nm with Molecular Devices plate reader (Sunnyvale, CA).

Total RNA preparation, cDNA synthesis, and real-time PCR

To determine whether $sigma_1$ receptor ligands regulate IL-10 mRNA expression, total splenocyte RNA was isolated using methods previously described (11). The reverse transcriptase reaction was performed with 500 ng total RNA with a kit from Life Technologies (Carlsbad, CA). For IL-10 mRNA determination, quantitative real-time PCR was performed using Amplifluor murine IL-10 direct gene system kit (Intergen, Purchase, NY) in an iCycler (Bio-Rad, Hercules, CA). The primers for the IL-10 PCR were: Amplifluor forward primer; 5′-CAT ACT GCT AAC CGA CTC CT-3′ and reverse primer; 5′-CTG GGG CAT CAC TTC TAC-3′. IL-10 cDNA provided in the kit was utilized to set up a standard curve. The PCR parameters were as follows: 94°C for 5 min (94°C for 15 s, 55°C for 40 s, 72°C for 1 min) × 45 cycles, 72°C for 3 min (final temperature, 25°C). The β-actin housekeeping gene was amplified to correct for RNA amounts using Amplifluor murine β-actin direct gene system (Intergen). The RT-PCR amplifications were examined by 2% agarose gel to rule out secondary bands. Only single bands were visible at the expected positions after EtBr staining of gels. Data analyses were performed using SigmaPlot (SPSS, Chicago, IL)

Lymphocyte transfer

Lymphocytes were isolated from spleens of the following groups of mice: tumor bearers, PRE-084-treated mice, tumor bearers receiving PRE-084, or diluent-treated BALB/c mice. Lymphocytes were isolated by using MACS microbeads in accordance with the manufacturer's protocol (Miltenyi Biotec, Auburn, CA). A total of 5×10^7 lymphocytes per injection were transferred to normal mice by lateral tail vein injection 1 day before and 7 days following inoculation of 10^5 L1C2 tumor cells. As a control, the same amount of L1C2 tumor cells were implanted in naive BALB/c mice without lymphocyte transfer. Tumor volumes were assessed three times per week.

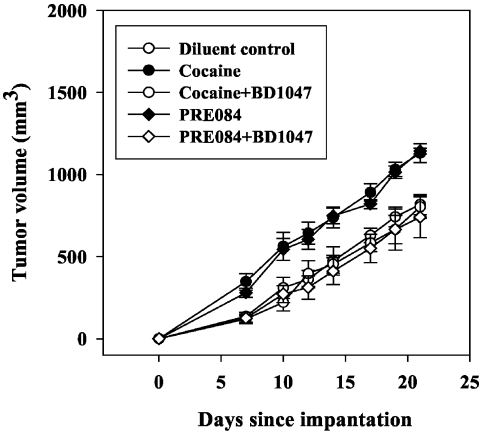

FIGURE 1. $Sigma_1$ receptor mediated promotion of tumor growth in vivo. BALB/c mice receiving i.p. injections of sigma receptor agonists cocaine (5 mg/kg), PRE-084 (20 mg/kg) or the agonists plus antagonists BD 1047 (20 mg/kg) for 2 wk were challenged with a s.c. injection of 10^5 L1C2 cells. Mice continued to receive the treatments for the duration of the experiment. There was a significant enhancement of L1C2 tumor growth in PRE-084 as well as in cocaine-treated mice ($p < 0.05$). The $sigma_1$ receptor agonist-mediated enhancement in tumor growth was ablated by treatment with BD1047 ($p < 0.05$). BD1047 treatment alone did not affect the tumor growth (data not shown) ($n = 8$–12 mice per group).

Results

$Sigma_1$ receptor agonists promote tumorigenicity in immunocompetent mice

To determine how $sigma_1$ receptor agonists modulate antitumor immune responses, we evaluated their effects on the growth of L1C2 tumors in vivo. L1C2 tumor cells were inoculated s.c. in PRE-084 or cocaine-treated BALB/c mice, and tumor growth was compared with that in diluent-treated control mice. As shown in Fig. 1, there was significant enhancement of L1C2 tumor growth in PRE-084 or cocaine-treated mice compared with control mice. These agonists acted in a sigma receptor-dependent manner as demonstrated by reversal of their tumor growth-promoting activity by BD1047, a sigma receptor antagonist. BD1047 treatment alone did not alter tumor growth (data not shown).

$Sigma_1$ receptor ligands modify tumor cytokine profiles in vivo

Based on previous studies demonstrating $sigma_1$ receptor modulation of cell-mediated immune responses (4, 5, 12, 13), we speculated that $sigma_1$ receptor ligands could be enhancing tumor growth by augmenting immune suppressive cytokine profiles in vivo. Non-necrotic tumors were isolated from L1C2 tumor-bearing BALB/c mice that had been treated with PRE-084 or cocaine as previously described and evaluated for IL-10, TGF-β, IFN-γ, and PGE_2 production. As shown in Table I, tumor homogenates from PRE-084- or cocaine-treated mice produced significantly more IL-10, TGF-β, and PGE_2 but less IFN-γ than did diluent-treated controls. Thus, $sigma_1$ ligand administration leads to a profile of increased immunosuppressive cytokines with enhanced IL-10, PGE_2, and TGF-β production but decreased IFN-γ release.

To determine the lymphocyte phenotype responsible for the production of IL-10 and TGF-β, we assessed the percentage of CD4 and CD8 T cells producing these cytokines at the tumor site. There was an increase in the percentage of both CD4 and CD8 T cells producing IL-10 in response to PRE-084 treatment. In contrast, there was no increase in CD4 and CD8 T cells producing TGF-β. However, the overall percentage of TGF-β-producing cells was increased in response to PRE-084. These findings suggest that

Table I. *The sigma$_1$ receptor agonist PRE084 and cocaine modulate cytokine production at the tumor site*[a]

	TGF-β (ng/ml/500 mg of tumor)	IL-10 (pg/ml/500 mg of tumor)	IFN-γ (pg/ml/500 mg of tumor)	PGE_2 (ng/ml/500 mg of tumor)
Diluent control	6.1 ± 1.6	58.0 ± 7.3	155.3 ± 16.8	11.9 ± 0.7
PRE 084 (20 mg/kg)	10.4 ± 0.7	435.0 ± 7.3	62.7 ± 6.8	25.7 ± 3.8
p value	<0.05	<0.01	<0.01	<0.01
Diluent control	2.9 ± 0.4	73.2 ± 7.1	86.4 ± 10.9	19.6 ± 0.6
Cocaine (5 mg/kg)	4.3 ± 0.3	128.8 ± 19.5	41.2 ± 2.5	33.1 ± 4.4
p value	<0.05	<0.05	<0.05	<0.05

[a] Non-necrotic tumors were isolated from the L1C2 tumor-bearing mice. Mice were treated with PRE 084, cocaine, or diluent control using the dosing and injection schedule as described above ($n = 6$ per group).

sigma$_1$ receptor agonists may induce TGF-β production from tumor cells or other nonlymphocyte host-cell populations in vivo.

Sigma$_1$ receptor-dependent modulation of cytokine production in vivo in non-tumor-bearing mice

To evaluate the sigma receptor-mediated production of cytokines independent of the influence of the tumor-bearing state, we assessed cytokine production following administration of PRE-084 or cocaine in non-tumor-bearing mice. Mice receiving injections of these sigma ligands were found to have significant induction of splenocyte IL-10 production and decreased IFN-γ release (Table II). Thus, there was a sigma receptor-mediated production of cytokines independent of the influence of the tumor-bearing state. However, splenocyte TGF-β production was not significantly altered by these treatments. To assess the capacity of sigma ligands to alter the type 1 cytokine profile in response to superantigens, cytokines were measured following SEB administration in BALB/c mice. Superantigens including SEB have been found to polyclonally activate T cells in vivo leading to secretion of cytokines, including IL-2 and IFN-γ (14). In contrast, tolerance to superantigen-induced cytokine production is mediated by IL-10 and TGF-β (15). Sigma ligands can potently modulate SEB-induced cytokine production in mice (16). Based on these previous findings, we hypothesized that sigma receptor ligation would induce a cytokine pattern consistent with tolerance to superantigen and therefore result in high level IL-10 production. In keeping with this postulate, following SEB administration in vivo, the level of IL-10 was significantly increased in cocaine-treated mice (Fig. 2). A specific sigma$_1$ receptor antagonist blocked this effect, thus documenting the sigma$_1$ receptor dependence. Thus cocaine mediates a sigma receptor-dependent induction of IL-10 that is maintained in the setting of a potent type 1 cytokine stimulus.

Table II. *Sigma$_1$ receptor-dependent modulation of cytokine production in splenocytes from nontumor-bearing mice*[a]

	IL-10 (pg/ml/2 × 10^6 cells)	IFN-γ (pg/ml/2 × 10^6 cells)	TGF-β (ng/ml/2 × 10^6 cells)
Diluent control	330 ± 29	658 ± 13	4 ± 0
PRE 084 (20 mg/kg, daily, 2 wk)	609 ± 50	244 ± 51	4 ± 0
p value	<0.01	<0.01	0.1
Cocaine (5 mg/kg, daily, 2 wk)	520 ± 29	342 ± 65	4 ± 0
p value	<0.05	<0.01	0.2

[a] To evaluate the sigma receptor-mediated production of cytokines independent of the influence of the tumor-bearing state, we assessed splenocyte cytokine production following administration of PRE 084 or cocaine in nontumor-bearing mice. Mice received injections of the sigma receptor ligands 5 days per week for 2 wk. IL-10, TGF-β, and IFN-γ levels were evaluated in cultured splenocytes by cytokine-specific ELISA ($n = 6$ per group).

Sigma receptor agonists induce IL-10 production in vitro

To determine whether sigma$_1$ receptor ligation directly up-regulates IL-10, BALB/c splenocytes were exposed to sigma$_1$-specific agonists in vitro. Consistent with the in vivo findings, we found that the sigma$_1$ receptor agonists PRE-084, SKF 10047, and cocaine induced IL-10 mRNA and protein production in splenocytes in vitro. In the presence of the sigma$_1$ receptor-specific antagonist BD1047, each of the agonists had significantly diminished IL-10 induction capacity (Fig. 3). BD1047 alone did not affect IL-10 levels (data not shown). Thus, sigma agonists significantly induce IL-10 in vitro and in vivo in a receptor-dependent manner. Consistent with G protein-coupled receptor signaling, the SKF 10047-, 4-IBP-, or cocaine-induced IL-10 production was significantly inhibited by PT (10 ng/ml). PT alone did not affect IL-10 production (Fig. 4). These findings indicate that sigma receptor agonists stimulate IL-10 production in a PT-sensitive manner.

Administration of anti-IL-10 mAb prevents the sigma$_1$ receptor agonist-mediated increase in tumor growth in vivo

IL-10 has been found to potently inhibit host immunity (17, 18) and may act at several points to interfere with either the generation or maintenance of antitumor immune responses. Based on the previously documented detrimental effects of IL-10, we speculated that sigma$_1$ receptor-mediated induction of IL-10 could be responsible for enhanced tumor growth in vivo. To determine the contribution of the heightened IL-10 production to the increased rate

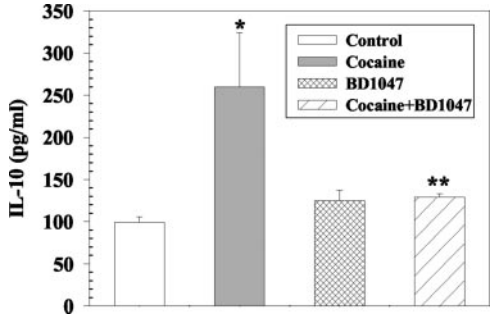

FIGURE 2. Sigma$_1$ receptor-dependent induction of IL-10 in vivo in response to SEB. BALB/c mice were pretreated five times per week for 2 wk with i.p. injections of cocaine (5 mg/kg), BD1047 (20 mg/kg), cocaine plus BD1047, or diluent control. The sigma$_1$ receptor antagonist, BD1047, was given 30 min in advance of agonist administration. SEB (50 μg/mouse) was administered via a lateral tail vein injection. Two hours following SEB injection, blood samples were obtained by retro-orbital puncture and IL-10 in the sera was quantified by ELISA. BD1047 significantly inhibited the capacity of cocaine to induce IL-10 ($n = 6$ mice per group). *, $p < 0.05$ compared with diluent control; **, $p < 0.05$ compared with cocaine.

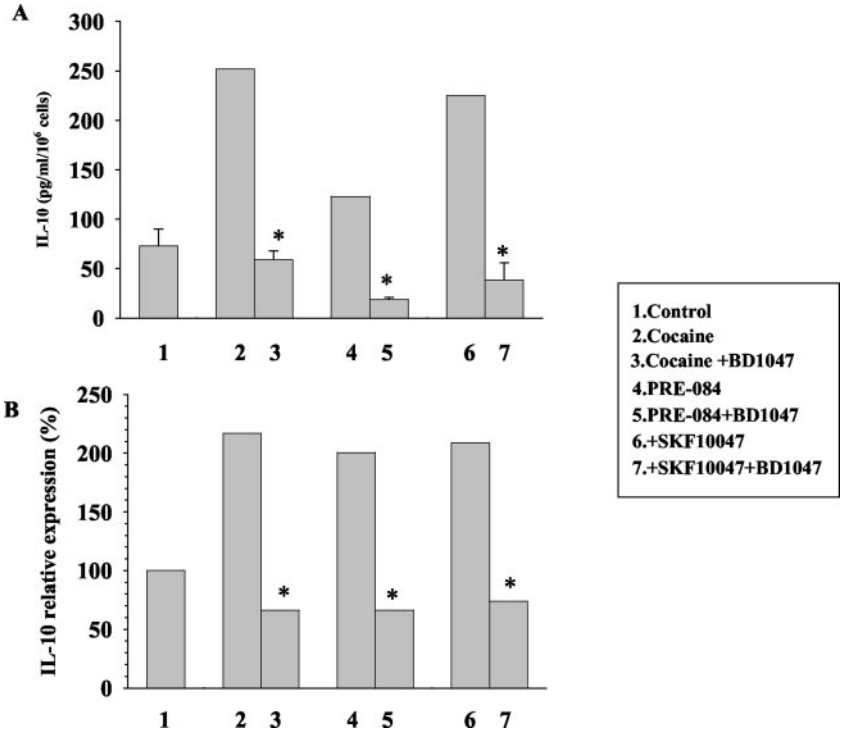

FIGURE 3. Sigma$_1$ receptor agonists induce splenocyte IL-10 mRNA and protein in vitro. *A*, BALB/c splenocytes were cultured with IL-2 (100 U/ml) for 3 days and then stimulated with sigma$_1$ agonists PRE-084 (0.3 μM), SKF 10047 (0.3 μM), or cocaine (0.3 μM) in the presence or absence of the sigma$_1$ receptor antagonist, BD1047 (0.3 μM), for 24 h. Sigma$_1$ receptor agonists induced splenic IL-10 production that could be completely blocked in the presence of the sigma$_1$ receptor antagonist BD1047 ($p < 0.05$). BD1047 treatment alone did not affect IL-10 production (data not shown). *B*, Total RNA was isolated from splenocytes following exposure to agonists and antagonists as in *A*. IL-10 mRNA expression was determined by quantitative real-time PCR. Sigma$_1$ receptor agonists, cocaine, PRE-084, and SKF 10047 potently induced splenic IL-10 mRNA that was blocked by the sigma$_1$ receptor antagonist BD1047 ($p < 0.05$).

of tumor growth in PRE-084- or cocaine-treated mice, anti-IL-10 mAb was administered to mice receiving PRE-084 or cocaine. Anti-IL-10 mAb, but not control Ab, prevented the sigma$_1$ receptor agonist-induced increase in tumor growth (Fig. 5, *A* and *B*).

Transfer of lymphocytes from sigma$_1$ receptor agonist-treated mice augments tumorigenicity in normal mice.

Because it appeared that sigma$_1$ receptor agonist mediated its immunosuppressive effects by up-regulating production of IL-10 from immune cells, we assessed the capacity of lymphocytes from sigma$_1$ receptor agonist-treated mice to transfer the immune deficit to normal control mice. Fifty million splenic lymphocytes from PRE-084-treated mice were transferred to normal mice by i.v. tail vein injection 1 day before and 7 days following s.c. inoculation of 10^5 L1C2 tumor cells. Following transfer of lymphocytes from PRE-084-treated mice to normal controls, the L1C2 tumor growth was augmented revealing a similar pattern to that demonstrated for transfer of lymphocytes from tumor-bearing mice. Tumor growth in mice receiving lymphocytes from PRE-084-treated tumor-bearing mice showed an even greater enhancement in tumor growth (Fig. 6). In contrast, transfer of lymphocytes from diluent-treated control mice to tumor-bearing control mice did not alter tumor growth.

Discussion

Sigma receptors have been identified as important high-affinity binding sites for a variety of drugs (1). Although initially identified in neuronal tissue, these receptors have also been found at other sites including cells of the immune system (19). The fact that sigma receptor expression has been noted in a variety of tissue sites has led to the suggestion that they may serve a broader biological role in mediating signaling well beyond their original description as neurotransmitter receptors (1). Previous studies indicate that sigma ligands can profoundly affect immune function (4, 5, 12, 20–23). In the present study we find that sigma ligands, including cocaine, can promote tumor growth by inducing the production of IL-10 in an immunocompetent murine model of lung cancer. Specific sigma$_1$ receptor antagonists abrogated the tumor growth promoting activities of cocaine, as well as its capacity to induce IL-10 in vitro.

Cocaine was used as a model sigma receptor agonist in our studies because, at clinically relevant concentrations, it has been previously documented to interact with sigma$_1$ receptors (24). Furthermore, specific sigma$_1$ receptor antagonists or antisense oligonucleotides have been found to block cocaine-induced behaviors (25–28). Relevant to our current investigations of sigma receptor-mediated modulation of

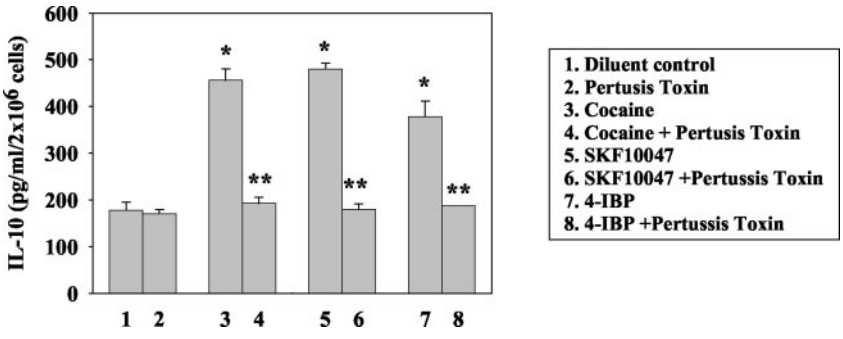

FIGURE 4. Sigma$_1$ receptor agonists induce IL-10 production in a PT-sensitive manner. BALB/c splenocytes were stimulated with IL-2 and treated with sigma$_1$ receptor agonists (0.3 μM) the presence or absence of PT (10 ng/ml, added 24 h before agonist treatment). PT consistently abrogated sigma$_1$ receptor agonist-mediated induction of IL-10 production. *, $p < 0.05$ compared with diluent control; **, $p < 0.05$ compared with cocaine, SKF 10047, or 4-IBP alone.

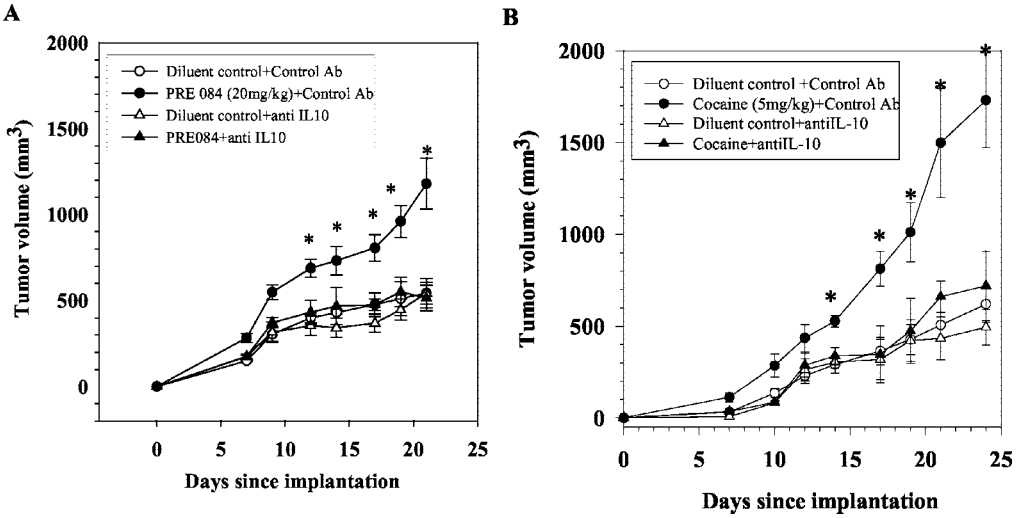

FIGURE 5. Administration of anti-IL-10 mAb prevents the sigma$_1$ receptor agonist or cocaine-mediated increase in tumor growth in vivo. Following treatment with sigma receptor agonists, BALB/c mice were injected with 10^5 L1C2 cells s.c. Anti-IL-10 Ab, JES-2A5, (5 mg/kg) or control Ab were administrated i.p. 1 day before tumor inoculation and then three times per week for the duration of the experiment. Mice continued to receive the sigma receptor agonists for the duration of the experiment. Anti-IL-10 mAb but not control Ab, prevented the PRE-084 or cocaine-induced increase in tumor growth ($p < 0.05$). The PRE-084 or cocaine-mediated increased tumor growth was not altered by control Ab treatment (data not shown) ($n = 8$–12 mice per group).

immune responses, cocaine has been found to interfere with host immunity (6, 29, 30). Cocaine has been shown to modulate cytokine production (31–33), alter the activity of CTLs and NK cells, and limit the B cell response to LPS (29). However, relatively little is known about the effects of cocaine on integrated immune responses such as the development of Ag-specific immunity or protection of the host against the challenge posed by infectious pathogens or tumor cells. In the current study, therefore, we sought to define the role of sigma$_1$ receptor ligation in mediating immunosuppression in the context of tumor growth in vivo.

Sigma$_1$ receptor ligation led to augmentation of IL-10 in a PT-sensitive manner suggesting that this network involves G-coupled protein signaling. However, based on reports in the literature, the precise relationship between sigma$_1$ receptors and PT-sensitive G protein coupled signaling is at present unclear. Because almost all G protein-associated receptors contain seven transmembrane regions, it has been suggested that sigma$_1$ receptors, which have only one transmembrane domain, may not be directly linked to G proteins (34). Sigma$_1$ receptors may, however, be associated with G proteins via indirect mechanisms (34). Sigma$_1$ receptor protein has been localized at both the endoplasmic reticulum as well as the nuclear envelope (35, 36), and the complex events leading to its documented activities will require further investigation.

The possibility that immune independent events play a role in sigma ligand regulation of tumor growth has been suggested by previous studies. Vilner et al. (37) have shown that both sigma receptor subtypes are highly expressed in tumor cell lines from various tissues, and Crawford and Bowen (38) reported that sigma$_2$ receptor agonists could activate apoptosis in breast tumor cells. In our present studies, sigma$_1$ receptor agonists did not affect murine L1C2 tumor cell proliferation or apoptosis in vitro at concentrations of 0.03–3 μM (data not shown). The disparity between our results and those of Crawford and Bowen (38) may be due to the difference in the cell lines evaluated or the distinct difference in the activities mediated by sigma$_1$ vs sigma$_2$ receptor ligation. Further studies will be necessary to define the importance of sigma receptor expression by tumor cells.

Previous studies suggest that populations of T cells in the tumor-bearing host may develop suppressor activities through the induction of IL-10 gene expression (17, 39). In accord with these studies documenting the importance of lymphocyte-derived IL-10 in the generation of tumor-induced tolerance, we speculated that sigma$_1$ receptor agonists might act to enhance tumor growth by promoting the induction of lymphocyte IL-10 in vivo. The fact that transfer of lymphocytes from PRE-084-treated mice had the capacity to transfer the immune deficit that led to enhanced tumor growth in normal control mice strongly supports this hypothesis.

Sigma$_1$ receptor agonists mediated an increase in tumorigenicity via up-regulation of IL-10. The ability of IL-10 to promote tumor growth is consistent with the known activities of this cytokine.

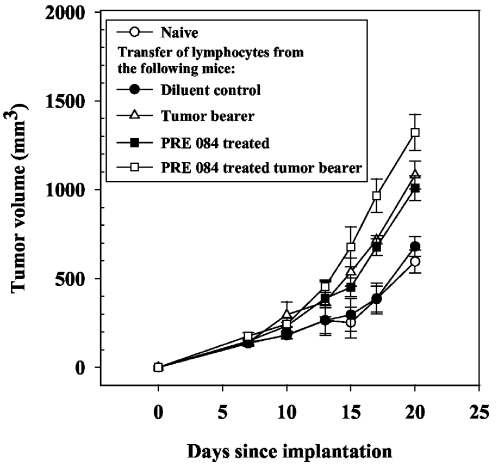

FIGURE 6. Transfer of lymphocytes from sigma$_1$ receptor agonist-treated mice augments L1C2 tumorigenicity in normal mice. BALB/c mice were pretreated for 2 wk with i.p. injections of PRE-084 (20 mg/kg) or diluent control. Lymphocytes were isolated from spleens of the following groups of mice: tumor bearers, PRE-084-treated mice, tumor bearers receiving PRE-084 or diluent-treated BALB/c mice. A total of 5×10^7 lymphocytes/injection were transferred to normal mice by a lateral tail vein injection 1 day before and 7 days following inoculation of 10^5 L1C2 tumor cells. As a control, naive BALB/c mice were implanted with the same amount of L1C2 tumor cells without lymphocyte transfer. Tumor volumes were assessed three times per week. ($n = 6$ mice per group).

IL-10 inhibits a broad array of immune parameters that include Ag presentation, Ag-specific T cell proliferation (40, 41), and type 1 cytokine production (42, 43). Pretreatment of tumor targets with IL-10 renders the tumor cells more resistant to CTL-mediated lysis (44, 45). We have previously found that production of IL-10 by cutaneous carcinomas provides a mechanism for evasion of the local T cell immune response (46). We also found that transgenic mice overexpressing IL-10 under the control of the IL-2 promoter were unable to limit the growth of immunogenic tumors (47). Administration of blocking IL-10 mAbs restored in vivo antitumor responses in these transgenic mice. These findings suggest that lymphocyte-derived and/or tumor-derived IL-10 production antagonizes antitumor immunity (17, 18, 48). The capacity for effector cells to generate cytokines is a critical element in the generation of effective immunity. Although the tumor-bearing state is accompanied by up-regulation of immune suppressive cytokines (49, 50), we speculated that $sigma_1$ receptor agonists could further augment the production of these deleterious cytokines. The host-immune response against tumors has been documented to be down-regulated by soluble mediators in the tumor environment (51). Tumors may either directly release factors or orchestrate immune suppressive networks by inducing host-immune cell production of inhibitory cytokines. IL-10 is an important immune inhibitory cytokine produced or induced by tumors causing limitations in immune reactivity against the tumor (51–53). In addition to IL-10, TGF-β, PGE_2, and IFN-γ are important mediators of immune responses that alter tumor growth. Elevated TGF-β leads to enhanced tumor growth by antagonizing CTL generation (54) and macrophage activities (55, 56). Increased PGE_2 can enhance tumor progression by limiting apoptosis, while increasing angiogenesis and invasiveness (57). IFN-γ is a type 1 cytokine that that promotes cell-mediated immunity and may therefore limit tumor growth. We found that $sigma_1$ receptor agonists increased the release of IL-10, TGF-β, and PGE_2, while decreasing IFN-γ at the tumor site, suggesting these agonists exaggerated tumor-induced immune suppression by regulating cytokine production. However, it appears that the increased TGF-β observed at the tumor site may be due to increased production by the tumor or tumor-induced cytokine production in host cells, because in the absence of tumor, $sigma_1$ receptor agonists did not increase the production of this cytokine in vivo. The fact that abrogation of IL-10 reversed the detrimental effects of $sigma_1$ receptor ligands strongly suggests that elevated IL-10 plays a significant role in mediating the $sigma_1$ receptor agonist-induced suppression of antitumor immunity. This is the first report of $sigma_1$ receptor-dependent alteration of antitumor immunity.

These findings may have important clinical implications for both medically prescribed drugs as well as those used in abuse. For example cocaine smokers have increased histopathologic abnormalities such as hyperplasia and squamous cell metaplasia compared with nonsmokers (58). In addition, cocaine smokers have elevated expression of molecular markers that have been associated with increased cancer risk such as Ki-67 and epidermal growth factor receptor (59). Thus cocaine smoking, like tobacco, has been implicated in field cancerization of bronchial epithelial cells (59). This leads to the speculation that the combination of these genetic alterations and sigma ligand-induced immune suppression may promote tumorigenesis. Further studies will be required to determine whether smoking cocaine causes cancer (60). Additional studies will also be necessary in tumor models and human cancer to define precisely both the details of the signaling events and the importance of these observations.

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