Cancer Immunol Immunother (1998) 46: 104–112

ORIGINAL ARTICLE

Charise M. White · Brian K. Martin · Li-Fen Lee
J. Stephen Haskill · Jenny P.-Y. Ting

Effects of paclitaxel on cytokine synthesis by unprimed human monocytes, T lymphocytes, and breast cancer cells

Received: 8 September 1997 / Accepted: 26 November 1997

Abstract Paclitaxel or Taxol has attracted a great deal of attention in recent years because of its immense success as a chemotherapeutic agent for numerous types of cancer. It is known that paclitaxel stabilizes microtubules, and this characteristic is the presumed primary mechanism for its antitumor activity. Recently, however, paclitaxel's ability to regulate gene expression, particularly in the murine system, has been reported by several groups. Here, we present research examining paclitaxel's ability to alter expression of the interleukin-1β (IL-1β) and IL-8 cytokines in primary human monocytes, T lymphocytes, and four human breast cancer cell lines: MCF-7, ZR-75-1, MDA-MB-468, and MDA-MB-231. This report shows for the first time that treatment with 5–50 μM paclitaxel increases steady-state levels of IL-1β mRNA in unprimed human monocytes, MCF-7, and ZR-75-1 cells. Monocytes from eight donors in 16 experiments showed increased IL-1β secretion upon treatment; however, the increase in IL-1β production by monocytes was predicated on culturing in the absence of fetal bovine serum or in the presence of autologous human serum. In contrast to the IL-1β results, paclitaxel did not have significant effects on IL-8 expression by monocytes, T lymphocytes, or the breast cancer cells. These data show a specific effect of paclitaxel on cytokine synthesis by both immune cells and cancer cells.

Key words Human · Monocytes/macrophages · T lymphocytes · Cytokines · Chemokines

This work was supported by NCI Grant and Minority Supplement CA48185.

C.M. White · J.S. Haskill · J.P.-Y. Ting
University of North Carolina at Chapel Hill, School of Medicine, Department of Microbiology and Immunology, Chapel Hill, North Carolina, USA

B.K. Martin · J.S. Haskill · J.P.-Y. Ting (✉)
Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, CB 7295, Chapel Hill, NC 27599-7295, USA
e-mail: panyun@med.unc.edu
Tel. +1 919 966 5538

L.-F. Lee
University of North Carolina at Chapel Hill, School of Medicine, Department of Biology, Chapel Hill, North Carolina, USA

J.S. Haskill
University of North Carolina at Chapel Hill, School of Medicine, Department of Obstetrics and Gynecology, Chapel Hill, North Carolina, USA

Introduction

Research in the field of cancer therapy has generated numerous treatments, which have had varying degrees of success. One of the most encouraging of these is paclitaxel (Taxol). Paclitaxel, the chemotherapeutic agent isolated from the bark of the Pacific yew tree (*Taxus brevifolia*), has shown promising results in the treatment of several types of cancer [17, 20]. Beneficial outcomes have been demonstrated in patients with malignant melanomas, non-small-cell lung carcinoma, and prostate cancer, but the most dramatic effects have been observed in clinical trials using paclitaxel in the treatment of ovarian and breast cancers [17, 20, 38]. These results were particularly encouraging because, in most cases, the tumors had been refractory to other methods of treatment [17].

Since the mid 1980s, a great deal of effort has been expended to determine why paclitaxel is such a potent tumoricidal agent. The primary mechanism by which paclitaxel kills cells is by preventing microtubular depolymerization [6, 16, 36, 37]. As a result, cells treated with paclitaxel are incapable of traversing the G2/M boundary of the cell cycle. They become growth-arrested and subsequently die by an apoptotic mechanism [2, 16, 18, 24].

While treatment with paclitaxel is capable of triggering programmed cell death in many tumor cells, it may exhibit pleiotropic effects on many different cells as well. Such effects may help explain why paclitaxel succeeds where other drugs, including colchicine and vinblastine, which also disrupt the normal assembly of microtubules, fail. Recently, attention has been given to the ability of paclitaxel to affect cytokine gene expression, particularly those

that are sensitive to lipopolysaccharide (LPS) [4, 25, 26]. The work presented here examines the response of three cell types: human primary monocytes, T lymphocytes, and breast cancer cell lines, to paclitaxel treatment. The focus is on the effect of paclitaxel on the expression of interleukin-1β (IL-1β) and/or IL-8, both of which are inducible by LPS.

We have chosen to investigate these cytokines for several reasons. IL-1β and IL-8 play significant roles in the immune response. One hypothesis for the increased efficacy of paclitaxel over other chemotherapies is that it triggers an immune response against the tumor. The IL-1β protein is made primarily by monocytes/macrophages, although the mRNA can be found in several cell types [11]. IL-1β is a potent mediator of local inflammation [12]. Encompassed in its myriad of effector functions are induction of other inflammatory cytokines, for example IL-2, IL-6, and IL-8, as well as cellular adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [45]. It also can enhance the phagocytic capabilities and major histocompatibility complex (MHC) class I antigen expression on monocytes/macrophages [29, 42]. IL-1β treatment causes neutrophils to release superoxide and, in hamsters, it has been shown to be a neutrophil chemoattractant [31, 33, 46]. IL-8 is also important in inflammation. Several cell types produce IL-8, including monocytes/macrophages, T cells neutrophils and some tumors [8, 21, 39, 44]. It was originally characterized by its ability to be a chemoattractant for neutrophils and has subsequently been shown to activate neutrophils to degranulate and release superoxides [14]. It may even be a chemoattractant for T cells, although there is currently some contention about this [7, 14, 22].

IL-1β and IL-8 have properties that can influence tumor growth. *In vitro* IL-1β can decrease the proliferation of endothelial cells and inhibit angiogenesis stimulated by basic fibroblast growth factor in the rabbit cornea [10]. By contrast, IL-8 strongly enhances angiogenesis [40]. In addition gene therapy experiments show direct effects of IL-1β on certain tumors culminating in either growth retardation or complete regression [3, 41].

The link between IL-1β, IL-8, and paclitaxel has been explored to some extent. Experiments performed using mouse macrophages have revealed that paclitaxel can increase the level of IL-1β mRNA in these cells [4]. However, similar experiments using human cells suggest that this is a species-specific phenomenon. In human cells, paclitaxel could act only in synergy with LPS to enhance IL-1β transcripts and secretion [1]. With regards to IL-8, a recent report from our group noted that paclitaxel induced IL-8 production and transcription in a subgroup of human ovarian cancer cell lines and freshly explanted tumors [21]. It is also known that the primary pathology observed after the clinical administration of paclitaxel is neutropenia [17]. Because of its effects on neutrophils, IL-8 may have a role in causing this neutropenia. This report reexamines the ability of paclitaxel to affect cytokine synthesis in human cells and demonstrates, for the first time, that paclitaxel can affect gene expression in human monocytes and breast cancer cells.

Materials and methods

Reagents

Paclitaxel was acquired through the generous donation of the National Cancer Institute (Bethesda, Md.) or purchased from Sigma (St. Louis, Mo.). In all of the experiments described, paclitaxel was used at concentrations ranging from 0.1 μM to 50 μM. This range is consistent with earlier studies using this agent. The paclitaxel was dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mM and stored at 4 °C or room temperature. Both the paclitaxel and DMSO were tested for the presence of contaminating LPS by the Lineberger Cancer Center Tissue Culture Facility (Chapel Hill, N.C.) using the *Limulus* amebocyte lysate test (BioWhittaker, Walkersville, Md.) and in all cases were found to have LPS levels below the lowest level of assay sensitivity, which was typically below 12.5 pg/ml. LPS (Sigma) and phytohemagglutinin (PHA) (Pharmacia Biotech, Piscataway, N.J.) were used as positive controls for monocytes and T lymphocytes respectively. Polymyxin B sulfate (Sigma) was used as an LPS inhibitor in some of the monocyte cultures.

Cell isolation and culture

Human primary monocytes and T lymphocytes were isolated from the peripheral blood of normal volunteers as previously described [5]. Briefly, peripheral blood was collected in the presence of 0.5 M ethylenediaminetetraacetic acid (EDTA). White blood cells were separated from red blood cells by centrifugation at 400 *g* in Histopaque-1077 (Sigma) cell separation medium. The white blood cell fraction then underwent several washes in 0.9% sodium chloride to remove platelets. To separate the monocyte and lymphocyte populations, the cells were centrifuged in a discontinuous Percoll (Phamacia) gradient [43]. Isolated monocytes were placed in RPMI-1640 medium (Life Technologies, Grand Island, N.Y.) supplemented with 20 mM L-glutamine (Life Technologies) and were used immediately in experiments. When fetal bovine serum (FBS; Hyclone Laboratories, Logan, Utah), gentamicin sulfate (Life Technologies), or HEPES (Life Technologies) was used, they were added at concentrations of 5% or 10%, 50 μg/ml, and 25 mM respectively. All of these reagents were tested for contaminating LPS as described earlier. No LPS was detected in any of these assays.

Immediately after isolation, monocytes were treated for 24 h under either adherent or non-adherent conditions. Cells cultured under adherent conditions were plated in either 60-mm or 24-well tissue-culture plates at a concentration of $(5–10)\times10^5$ cells/ml, and incubated at 37 °C in a 5% CO_2 incubator while those treated under non-adherent conditions were placed in either 6-ml polypropylene tubes or 1.7-ml microfuge tubes and incubated at 37 °C with continuous movement [23].

Lymphocytes were cultured in RPMI-1640 medium supplemented with 10% FBS (Hyclone Laboratories), essential and non-essential amino acids (Life Technologies) and 50 μg/ml gentamicin sulfate (Life Technologies) for 1 week to allow for the elimination of residual adherent cells and B cells. This procedure resulted in a population of T lymphocytes that was more than 95% $CD3^+$. Following this enrichment period, $(2–5)\times10^6$ cells/ml were treated with paclitaxel and incubated for 24 h at 37 °C in a 5% CO_2 incubator. All of the experiments shown here using T lymphocytes were performed in complete medium containing FBS.

The human breast cancer cell lines MCF-7, ZR-75-1, MDA-MB-231, MDA-MB-468 were obtained form the American Type Culture Collection (ATCC, Rockville, Md.) and were maintained and treated in RPMI-1640 medium supplemented with 7% fetal bovine serum (Life Technologies).

Enzyme-linked immunosorbant assay (ELISA)

Microtiter plates were coated overnight at 4 °C with 100 μl 5-μg/ml solution of a goat polyclonal antibody to either human IL-1β or human

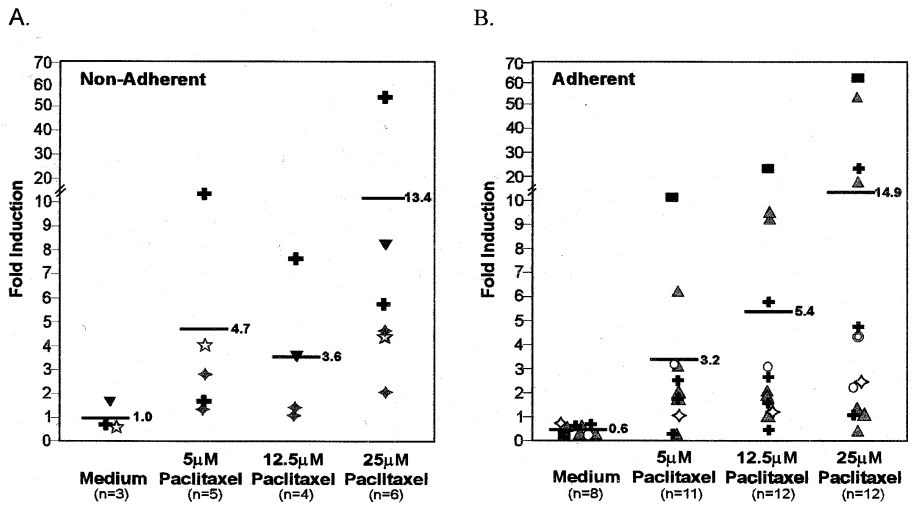

Fig. 1A, B Paclitaxel increases interleukin-1β (IL-1β) protein in human primary monocytes. Monocytes were isolated from the peripheral blood of healthy donors. In these experiments, eight different donors were used (each symbol represents a donor) for the 16 experiments performed. Cells were cultured in medium, in the presence of paclitaxel at the indicated concentrations or with dimethylsulfoxide (DMSO), under either non-adherent (**A**) or adherent (**B**) conditions for 24 h. Supernates were collected and examined for IL-1β by enzyme-linked immunosorbent assay (ELISA). The data are presented as fold induction over the DMSO control. *Horizontal bars,* the mean fold induction for each group

IL-8 (R&D Systems, Minneapolis, Minn.) in bicarbonate buffer (1.59 mg/ml Na_2CO_3, 2.93 mg/ml $NaHCO_3$, 0.2 mg/ml NaN_3). The plates were washed with phosphate-buffered saline (PBS) + 0.05% Tween 20 and blocked in 200 µl blocking buffer (20 mg/ml bovine serum albumin, 0.5 NaN_3, and PBS) for 2 h at 37 °C. Following removal of blocking buffer, 100 µl serially diluted recombinant human IL-1β or IL-8 (R&D Systems), which served as a concentration standard, or 100 µl cell culture supernate was added and allowed to incubate overnight at 4 °C. The plates were washed 16–24 h later and a second cytokine-specific antibody was added at a concentration of 1 µg/ml. The antibodies for the IL-1β and the IL-8 ELISA were mouse anti-(human IL-1β) (R&D Systems) and rabbit anti-(human IL-8) (Endogen, Boston, Mass.). After a 2-h room-temperature incubation, the plates were washed, and 1 µg/ml alkaline-phosphatase-conjugated secondary antibody was added [either goat anti-(mouse IgG) (Sigma) or goat anti-(rabbit IgG) (Cappel, Durham, N.C.)]. The plates were left at room temperature for an additional 2 h, washed, and incubated with 100 µl substrate *p*-nitrophenylphosphate (1 mg/ml) (Bio-Rad, Hercules, Calif.). The reaction proceeded at room temperature until a color change was observed, at which time the absorbance of the standard and samples was read at a wavelength of 405 nm. The standards were used to generate a curve (Cricket Graph or Jandel Sigma Plot computer software) from which the concentration of cytokine in the samples was interpolated.

Northern blot analysis

Total RNA was isolated from monocytes using the Trizol reagent (Life Technologies) adhering to the manufacturer's protocol. The isolated RNA was resuspended in diethylpyrocarbonate-treated water. Up to 8 µg RNA, as determined by optical absorbance at 260 nm, was diluted in denaturing mix (Current Protocols in Molecular Biology) at a ratio of 1:2. The samples were heated at 65 °C for 30 min and electrophoresed in a denaturing agarose gel. Subsequently, the RNA was transferred to nylon membrane, and probed with a ^{32}P-radiolabeled probe encompassing 250 bases of the IL-1β gene, or approximately 700 bases of the rat cyclophilin gene, and examined by autoradiography. Quantification was performed using the NIH Image computer software.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from primary T cells via the Trizol (Life Technologies) reagent and from the breast cancer cell lines using guanidine isothiocyanate cell lysis followed by centrifugation in a cesium chloride gradient. cDNA was prepared using the Life Technologies M-MLV reverse transcriptase. Briefly, 10 µg RNA was incubated at 37 °C for 2 h with 400 U Moloney murine leukemia virus (M-MLV) reverse transcriptase, 0.5 µg random hexamer primers, and 1.25 mM dNTP. For PCR amplification, 200 ng cDNA was mixed with Taq reaction buffer (0.4 U Taq DNA polymerase and buffer (Life Technologies), 1.25 mM of each of the dNTP, trace amounts of [α-^{32}P]dCTP and forward and reverse primers specific for sequences within either the IL-1β (5′-AAGATGAAGGGAAAGAAGGTG and 5′-TACAGTGGCAATGACGATGAC), IL-8 (5′-TCTGCAGCTCTGTGTGAAGG TGCAGTT and 5′-ACCCCTCTGCACCCAGTTTTCCTT), or cyclophilin (5′-GGTTTATGTGTCAGGGTGGTG and 5′-TTCATGCCTTCTTTCACTTTG) gene. The sequence was amplified for 20–30 cycles in a thermal cycler (Perkin Elmer Cetus, Branchburg, N.J.). One cycle consisted of a 1-s, 94 °C denaturing step; a 1-s, 60 °C annealing step; and a 20-s, 72 °C extension step. Products were then separated by electrophoresis in a 6% denaturing polyacrylamide gel and examined by autoradiography.

Results

Paclitaxel stimulates human primary monocytes to secrete IL-1β in a dose-dependent fashion

ELISA were performed to determine whether paclitaxel alone could stimulate the production of IL-1β in human monocytes/macrophages. Peripheral monocytes were taken from normal donors and exposed to either 25, 12.5, or 5 µM paclitaxel for 24 h. These concentrations are within the range used in other studies. Control cells were left untreated or treated with DMSO, which served as the solvent for the paclitaxel. After 24 h, there was no increased cell death, as determined by trypan blue exclusion, in the paclitaxel-treated samples compared to the control. Medium harvested from the cultures was tested for the presence of IL-1β by

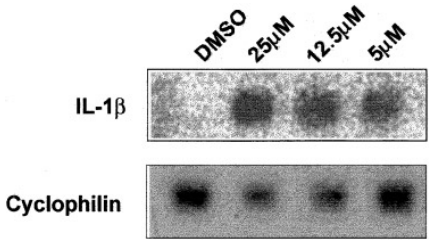

Fig. 2 Paclitaxel increases steady-state levels of IL-1β mRNA in umprimed human monocytes. Total RNA was harvested from approximately 2.75×10^6 human peripheral blood monocytes, which were treated with either paclitaxel (25 μM, 12.5 μM, and 5 μM) or DMSO (control) for 24 h under adherent conditions. The RNA was analyzed for IL-1β transcripts by Northern blot analysis

Table 1 Induction (-fold) of interleukin-1β (IL-1β) mRNA and protein secretion in paclitaxel-treated monocytes. mRNA was quantified by NIH Image analysis; IL-1β protein secretion was determined by enzyme-linked immunosorbent assay

[Paclitaxel] (μM)	mRNA (-fold)	IL-1β (ng/ml)
25.0	13.8	63.3
12.5	10.8	25.3
5.0	6.4	12.3

ELISA. The data were standardized to the concentration of IL-1β produced by control cells treated with DMSO alone; consequently, they are presented as -fold inductions over the DMSO control (Fig. 1). Figure 1A is a compilation of the data from experiments in which the cells were incubated under non-adherent conditions, while Fig. 1B represents those performed under adherent conditions. This experiment was conducted 16 times using peripheral blood from eight different donors. At each of the concentrations tested, paclitaxel stimulated IL-1β production. The 25 μM concentration gave the greatest mean induction: 13.4 and 16.9 for the non-adherent and adherent groups respectively. The lower concentrations of 12.5 μM and 5 μM also induced elevated secretion of the cytokine giving inductions of 3.6- and 4.7- (non-adherent) and 5.8- and 3.5-fold (adherent).

IL-1β mRNA is increased in paclitaxel-treated monocytes in a dose-dependent fashion

To determine whether paclitaxel treatment altered the level of IL-1β mRNA in monocytes, primary monocytes were cultured with 5, 12.5, or 25 μM paclitaxel. Total RNA and supernatant medium were collected from the cultures 24 h later. The RNA was examined for IL-1β by Northern analysis (Fig. 2). The data were quantified and adjusted for uneven mRNA loading as determined by the cyclophilin signal. These results are shown in Table 1 as -fold induction over the DMSO signal. The supernates from these same cells were examined for IL-1β protein by ELISA. These results also are shown in Table 1. The data clearly show a correlation between increased IL-1β mRNA and increased

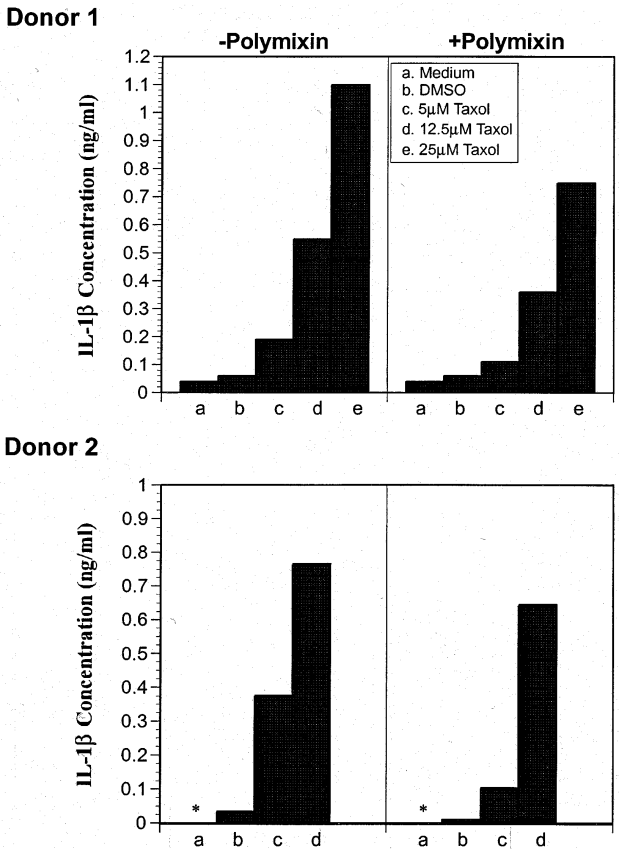

Fig. 3 Increased IL-1β production is not due to lipopolysaccharide (LPS). Monocytes from two donors were left untreated (*a*), or treated with DMSO (*b*) or with 5 μM (*c*), 12.5 μM (*d*), or 25 μM (*e*) paclitaxel (Taxol) in the absence or presence of polymixin B sulfate. Cells from donor 1 and donor 2 were treated with 200 pg/ml and 1 ng/ml polymixin B sulfate respectively. The cells were cultured on plastic tissue-culture plates at a concentration of about 6×10^5 monocytes/ml. After 24 h, the supernates were collected and analyzed for IL-1β by ELISA*. Samples that were below the minimum detection limit of the assay

protein secretion. The dose-dependent response observed at the protein level is reflected at the mRNA level as well, suggesting that elevations in IL-1β mRNA upon paclitaxel treatment are likely to contribute to increased IL-1β secretion.

Fetal bovine serum inhibits paclitaxel-induced IL-1β increase in human monocytes

Previous research using human cells suggested that paclitaxel alone does not stimulate human macrophages to increase IL-1β secretion [1]. Because our results contradict this, it was necessary to explore reasons for these differing results.

A potential explanation for the results reported here is that IL-1β induction is due to LPS contamination of the culture system. If this were indeed the case, then the enhancement observed could be attributed to synergy between LPS and paclitaxel. Several precautions were taken

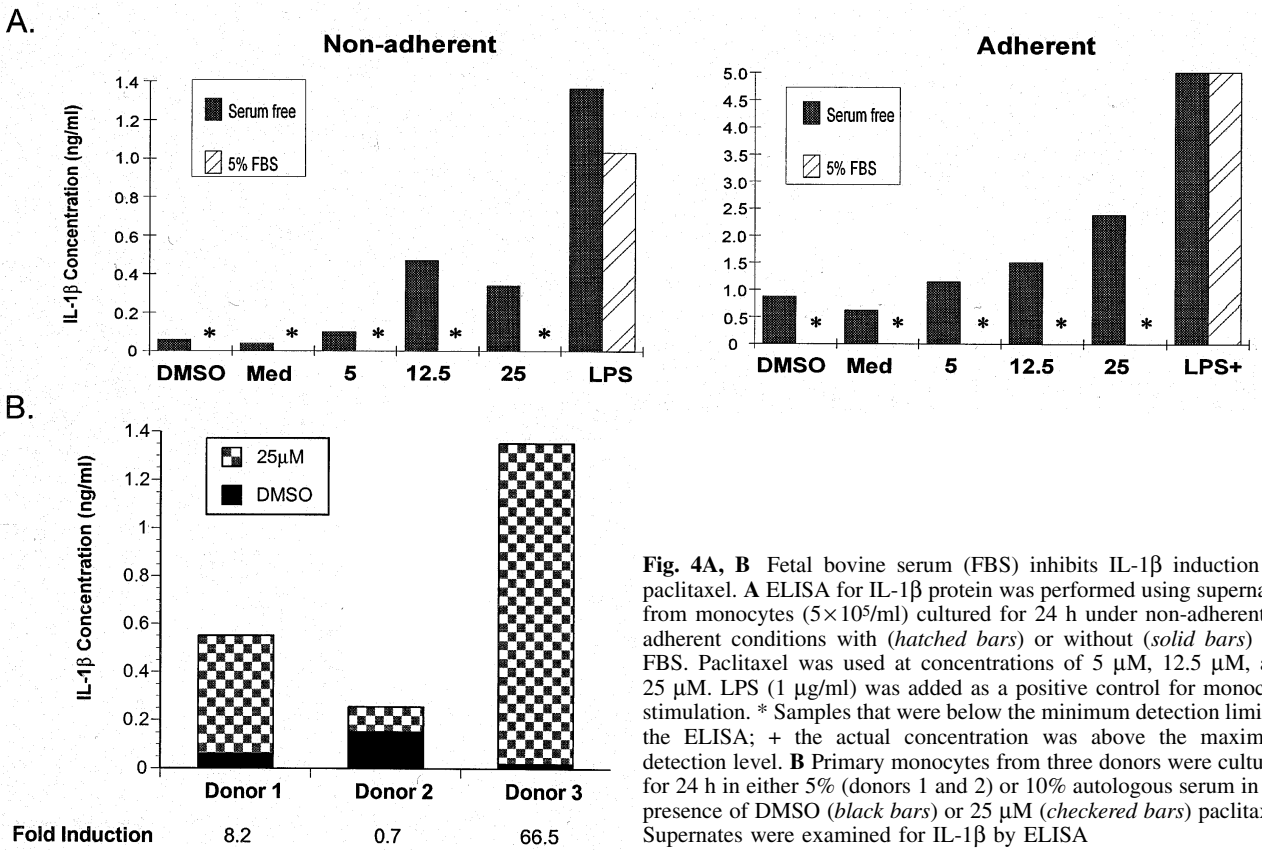

Fig. 4A, B Fetal bovine serum (FBS) inhibits IL-1β induction by paclitaxel. **A** ELISA for IL-1β protein was performed using supernates from monocytes (5×10^5/ml) cultured for 24 h under non-adherent or adherent conditions with (*hatched bars*) or without (*solid bars*) 5% FBS. Paclitaxel was used at concentrations of 5 μM, 12.5 μM, and 25 μM. LPS (1 μg/ml) was added as a positive control for monocyte stimulation. * Samples that were below the minimum detection limit of the ELISA; + the actual concentration was above the maximum detection level. **B** Primary monocytes from three donors were cultured for 24 h in either 5% (donors 1 and 2) or 10% autologous serum in the presence of DMSO (*black bars*) or 25 μM (*checkered bars*) paclitaxel. Supernates were examined for IL-1β by ELISA

to minimize this possibility. The medium, paclitaxel, and DMSO were all tested for the presence of LPS. The test results confirmed that any LPS in the medium used were at levels below the lower detection limit of the *Limulus* amebocyte assay (BioWhittaker) (<12.5 pg/ml). In addition, three experiments (the results from two are shown in Fig. 3) were done in the presence of a great excess of polymyxin B sulfate, which binds to LPS and inhibits its ability to activate cells presumably by interfering with binding to CD14, the LPS receptor [9]. The *Limulus* amebocyte assay determined that the LPS contamination in the culture medium, if present, was less than 12.5 pg/ml. We empirically determined that a 20:1 ratio of polymyxin to LPS could block all of the LPS- stimulated IL-1β induction. The cells from donor 1 were incubated with a 20-fold excess of polymyxin in conjunction with paclitaxel or DMSO, assuming that 10 pg/ml was the level of contamination, while those of donor 2 were in the presence of a 100-fold excess. In both experiments, paclitaxel induced IL-1β secretion beyond the DMSO control despite the presence of a great excess of the LPS inhibitor. The fact that the IL-1β protein concentration was slightly decreased in some of the polymyxin-treated samples is likely attributable to polymyxin's ability to inhibit protein kinase C. The protein kinase C signal-transduction pathway has been implicated in the paclitaxel-induced production of tumor necrosis factor α (TNFα) observed in mouse macrophages, and in both IL-1β and TNFα induction by *Toxoplasma gondii* [15, 19].

We next examined the possibility that variances in methodologies caused the differing results. One variable already shown is the utilization of nonadherent rather than adherent culture conditions. Other investigations used only adherent conditions. Figure 1 shows there is no significant difference between the mean -fold inductions of IL-1β secretion between the non-adherent and adherent groups.

Another major difference between the experiments described here and previous work using human monocytes is that the cells used here were cultured in the absence of FBS. Figure 4A represents one experiment comparing the effect of FBS on adherent and non-adherent cultures. These experiments have been performed with four additional donor samples yielding a similar trend. The addition of serum to non-adherent or adherent cultures not only inhibited the paclitaxel-induced enhancement of IL-1β but, in many cases, also diminished the amount of IL-1β secretion to levels below the sensitivity of the ELISA. LPS overcame the inhibitory effect of FBS, but paclitaxel did not. While the underlying mechanism is unclear, this may explain the discrepancy between our data and that previously published. Autologous serum, however, did not yield the same negative effect. Cultures supplemented with 5% (donors 1 and 2) or 10% (donor 3) autologous serum showed an induction pattern similar to that observed in Fig. 1 (Fig. 4B). In two of the three donors examined, paclitaxel significantly increased secretion of IL-1β. In the other donor, paclitaxel caused a less dramatic but detectable increase. This suggests that the inhibition observed in the presence of FBS

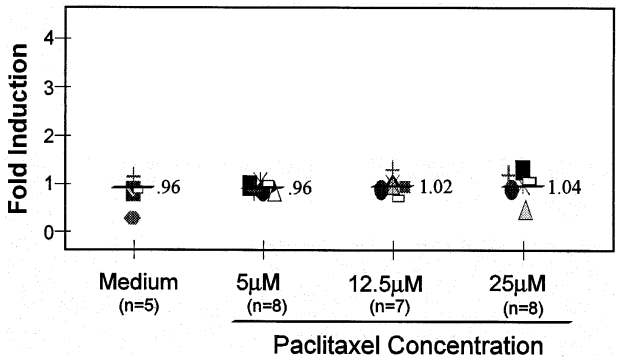

Fig. 5 IL-8 production in monocytes is not increased by paclitaxel. Primary monocytes cultured under non-adherent or adherent conditions were incubated for 24 h. The cells were either left untreated (*medium*) or treated with paclitaxel at the indicated concentrations or with DMSO. After the incubation, supernates from the cultures were analyzed for IL-1β protein by ELISA. Four different donors (different symbols) provided monocytes for the eight experiments. *Horizontal lines,* the mean -fold induction other the DMSO control

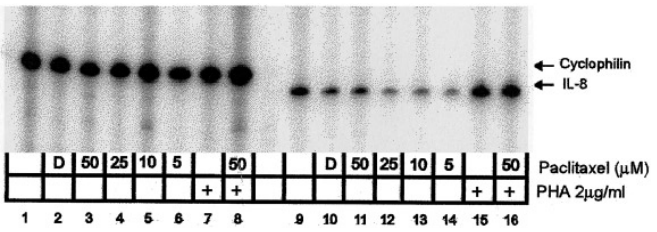

Fig. 6 Paclitaxel has minimal effects on IL-8 production by human, primary T lymphocytes. Total RNA was isolated from T lymphocytes following a 24-h incubation with paclitaxel at concentrations ranging from 5 μM to 50 μM, DMSO, and/or phytohemagglutinin (*PHA*; 2 μg/ml). The amount of IL-8 mRNA was measured using a semi-quantitative reverse transcriptase/polymerase chain reaction (RT-PCR). *Lanes 1–8* cyclophilin; *Lanes 9–16* IL-8

is specific and not a general characteristic of all serum. The fact that the induction can be observed in the presence of human serum also indicates that it is possible that paclitaxel stimulates IL-1β production in vivo.

IL-8 expression in human primary monocytes and T lymphocytes is not affected by paclitaxel treatment

We previously reported that paclitaxel induces IL-8 mRNA and secretion in certain human ovarian carcinomas [21]. To determine whether this induction is limited to those cells or whether it is specific for the IL-8 gene regardless of cell type, paclitaxel-treated monocytes were examined for IL-8 protein secretion by ELISA. Four different donors provided monocytes for the eight experiments represented in Fig. 5. All of these experiments were conducted in serum-free medium. The mean -fold inductions of the experimental groups treated with paclitaxel and the DMSO control were indistinguishable. Paclitaxel did not modify IL-8 protein levels at any of the concentrations tested.

T lymphocytes are another source of IL-8 in humans. To examine the effect of paclitaxel on IL-8 expression in these cells, RNA from paclitaxel-treated T lymphocytes cultured in complete T cell medium was isolated and analyzed for the presence of IL-8 mRNA (Fig. 6). The first eight lanes depict the amount of cyclophilin present in each sample. Lanes 9–16 show the levels of IL-8 mRNA. Control reactions performed in the absence of cDNA were negative for any signals (data not shown). T lymphocytes treated with either 5, 10, or 25 μM paclitaxel (lanes 12, 13, and 14) did not show an increase in IL-8 message after a 24-h treatment. In fact there was a slight decrease at all concentrations except for 50 μM when compared to the DMSO-treated control (lane 10). To corroborate our findings, IL-8 secretion was analyzed by ELISA. The amount of IL-8 released mirrored the results from the RT-PCR (data not shown). No increase was observed at any paclitaxel concentration used. After culture for 24 h, none of the samples showed signs of proliferation; however, an increase in IL-8 was observed in the cultures treated with the T cell mitogen PHA (Fig. 6, lanes 15 and 16) indicating that 24 h is sufficient time to stimulate IL-8 production in T cells. The experiment shown in Fig. 6 was done in the presence of FBS. In others performed without it, no IL-8 protein was detected after treatment with paclitaxel, and the cells were resistant to induction by PHA.

Paclitaxel treatment increases IL-1β transcripts in a subgroup of breast cancer cell lines but has no effect on IL-8

Our previous results with ovarian cancer cell lines suggest that paclitaxel may induce cytokine expression in the cancer cells themselves [21]. To determine the effect of paclitaxel on IL-1β expression, we tested several breast carcinoma cell lines for mRNA and protein induction. As with monocytes, some breast cancer cell lines also responded to paclitaxel by increasing the steady-state level of IL-1β mRNA present in the cells (Fig. 7). In the estrogen-recept(ER)-positive cell line MCF-7, paclitaxol increased IL-1β in a dose-dependent manner. This reached a maximum of approximately 25-fold induction over the DMSO control at 25 μM paclitaxel. In a second ER-positive cell line, ZR-75-1, paclitaxel also induced IL-1β mRNA, but to a much lesser extent (a maximum of 5-fold; data not shown). Analysis of two ER-negative cell lines, MDA-MB-468 and MDA-MB-231, showed no induction. These data demonstrate that paclitaxel is able to induce IL-1β mRNA expression in some breast cancer cell lines. A more extensive survey is necessary to determine if the differential effects on ER-negative and -positive lines are significant.

None of these breast cancer cell lines induced IL-8 mRNA in response to paclitaxel treatment, nor could they be induced to secrete IL-8 protein in response to paclitaxel treatment. The results are not shown because they are largely negative data.

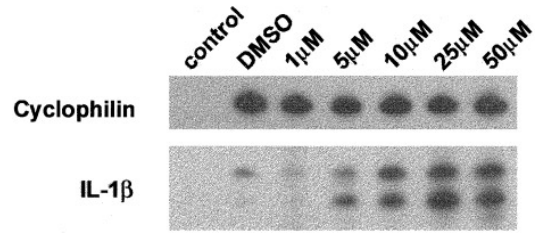

Fig. 7 Paclitaxel increases the level of ILl-1β mRNA in MCF-7 breast cancer cells. Semi-quantitative RT-PCR was performed using RNA from MCF-7 estrogen-receptor-positive breast cancer cells. The cells were treated with either DMSO or paclitaxel at concentrations ranging from 1 μM to 50 μM for 24 h. The RNA was examined for IL-1β and cyclophilin transcripts. Cyclophilin was used to standardize lane loading. The control lane does not contain cDNA

Discussion

Since 1971, when paclitaxel was purified from the Pacific yew tree, diligent research has proven that it has beneficial indications in the treatment of many tumor types including breast and ovarian cancers [17, 20]. It is clear that paclitaxel stabilizes microtubules, and this stabilization can result in cell death [6, 16, 37]. However, other consequences of paclitaxel treatment on both tumor and non-tumor cells have only recently been documented. Most notable are the effects of paclitaxel on cytokine production. The goal of this work was to investigate the effect of paclitaxel treatment on two human leukocyte populations, monocytes/macrophages and T lymphocytes, and a primary therapeutic target of paclitaxel, breast cancer cells, focusing on its ability to regulate expression of the IL-1β and IL-8 cytokines. A large sampling of human primary monocytes and four breast cancer cell lines (MCF-7, ZR-75-1, MDA-MB-468, and MDA-MB-231) were examined for altered IL-1β production following paclitaxel treatment. These cell types, as well as human primary T lymphocytes, were also examined for changes is IL-8 expression in response to paclitaxel. Surprisingly, in monocytes, paclitaxel alone elicits an increase in IL-1β secretion and mRNA. The in vitro culturing conditions appear to be critical for this induction as the absence of nonhuman serum is required for observing the IL-1β increase. Thus, the use of bovine serum in other studies may have obscured the effects of paclitaxel. Estrogen-receptor-positive breast cancer cell lines (ZR-75-1 and MCF-7) are also shown to induce IL-1β mRNA. Unlike IL-1β, paclitaxel treatment does not increase IL-8 secretion by monocytes, breast cancer cell lines, or T lymphocytes, and in fact it may have had a slight adverse affect on its synthesis in T lymphocytes. These studies suggest a pattern of complex cell-specific effects of paclitaxel treatment which may have implications for its therapeutic usage.

We show that paclitaxel increases steady-state IL-1β mRNA levels, but not IL-8, which suggests that the IL-1β enhancement is a gene-specific effect. There are few published data addressing paclitaxel's effect on transcriptional or post-transcriptional mRNA regulation. In the last few years, a substantial argument has been made for linkage of the paclitaxel signaling pathway to that of LPS in both the human and murine systems. A report by Perera et al. described how paclitaxel could induce the translocation of the transcriptional activator NF-κB to the nucleus of murine macrophages in a manner mimicking LPS [34]. In the nucleus, NF-κB could have a role in activating the IL-1β promoter. However, NF-κB is also found in the IL-8 promoter, but no increase was seen in IL-8 production with paclitaxel treatment. This suggests that, if the increase is transcription-mediated, paclitaxel affects other transcription factors or the other factors within the IL-1β promoter make it more sensitive to NF-κB. Also, the IL-1β transcript contains A+U-rich sequence elements, indicative of genes that can be post-transcriptionally controlled. Interferon-γ-enhanced LPS stimulation increases IL-1β mRNA by elevating transcription and mRNA stability; however, the evocation of this dual mechanism of mRNA increase is dependent upon the stimulus [13, 32]. Paclitaxel treatment does result in the stabilization of granulocyte/macrophage-colony-stimulating factor RNA in murine B cells [35]. In addition, a role for paclitaxel in the translation or post-translational modification of IL-1β is not inconceivable. By altering the cytoskeletal architecture within cells, paclitaxel may activate an intricate cascade of events influencing transcription, post-transcription, translation, and post-translational modifications.

Results of monitoring the effect of paclitaxel on human primary T lymphocytes are also shown. The data show that paclitaxel did not enhance IL-8 levels in these cells, and may even cause a down-regulation of its production. One of the few published reports addressing how T lymphocytes respond to paclitaxel treatment stated that paclitaxel could inhibit the cytotoxic effector functions of T cells [30]. Whether paclitaxel causes a general down-regulation of these cells or whether the IL-8 down-regulation observed here is a specific phenomenon will require further study.

Paclitaxel's place as an effective chemotherapeutic has been firmly established. It has been found to be effective in cases where other microtubule-affecting drugs have not. Taking into consideration the evidence presented here that paclitaxel causes IL-1β production by human monocytes and some tumor cells, one could postulate that this effect may contribute to its greater efficacy. There are numerous ways in which cytokines could be deleterious to tumors. For example IL-1β could initiate an inflammatory response against the tumor or impair vascularization of the tumor, which would restrict its growth. Currently studies are underway to determine the most beneficial route of paclitaxel administration for the treatment of ovarian cancer, intravenously or intraperitoneally [27, 28]. Intraperitoneal administration would expose peritoneal monocytes to high concentrations of paclitaxel which, as our results show, could increase IL-1β synthesis, aiding in tumor regression. This model will require testing, but it is clear that understanding the effects of paclitaxel on cells of the immune system and within the tumor will help improve the drug's efficacy.

Acknowledgements We thank the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute for generously donating the paclitaxel used in this research.

References

1. Allen JN, Moore SA, Wewers MD (1993) Taxol enhances but does not induce interleukin-1 beta and tumor necrosis factor-alpha production. J Lab Clin Med 122:374–381
2. Bhalla K, Ibrado AM, Tourkina E, Tang C, Mahoney ME, Huang Y (1993) Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukemia 7:563–568
3. Bjorkdahl O, Wingren AG, Hedhund G, Ohlsson L, Dohlsten M (1997) Gene transfer of a hybrid interleukin-1 beta gene to B16 mouse melanoma recruits leucocyte subsets and reduces tumour growth in vivo (in Process Citation). Cancer Immunol Immunother 44:273–281
4. Bogdan C, Ding A (1992) Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor alpha and interleukin-1 in macrophages. J Leukoc Biol 52:119–121
5. Boyum A (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 97:77–89
6. Caplow M, Shanks J, Ruhlen R (1994) How taxol modulates microtubule disassembly. J Biol Chem 269:23399–23402
7. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA (1994) Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc Natl Acad Sci USA 91:3652–3656
8. Colasante A, Mascetra N, Brunetti M, Lattanzio G, Diodoro M, Caltagirone S, Musiani P, Aiello FB (1997) Transforming growth factor beta 1, interleukin-8 and interleukin-1, in non-small-cell lung tumors (in Process Citation). Am J Respir Crit Care Med 156:968–973
9. Cooperstock MS (1974) Inactivation of endotoxin by polymyxin B. Antimicrob Agents Chemothe 6:422–425
10. Cozzolino F, Torcia M, Aldinucci D, Ziche M, Almerigogna F, Bani D, Stern DM (1990) Interleukin 1 is an autocrine regulator of human endothelial cell growth. Proc Natl Acad Sci USA 87:6487–6491
11. Dinarello CA (1991) Interleukin-1 and interleukin-1 antagonism. Blood 77:1627–1652
12. Dinarello CA (1993) Modalities for reducing interleukin 1 activity in disease (review). Immunol Today 14:260–264
13. Fenton MJ (1992) Review: transcriptional and post-transcriptional regulation of interleukin 1 gene expression (review). Int J Immunopharmacol 14:401–411
14. Graves DT, Jiang Y (1995) Chemokines, a family of chemotactic cytokines (review). Crit Rev Oral Biol Med 6:109–118
15. Grunvald E, Chiaramonte M, Hieny S, Wysocka M, Trinchieri G, Vogel SN, Gazzinelli RT, Sher A (1996) Biochemical characterization and protein kinase C dependency of monokine-inducing activities of Toxoplasma gondii. Infect Immun 64:2010–2018
16. Horwitz SB (1994) Taxol (paclitaxel): mechanisms of action (review). Ann Oncol 5 [Suppl 6]:S3–6
17. Huizing MT, Misser VH, Pieters RC, Bokkel Huinink WW ten, Veenhof CH, Vermorken JB, Pinedo HM, Beijnen JH (1995) Taxanes: a new class of antitumor agents (review). Cancer Invest 13:381–404
18. Jordan MA, Toso RJ, Thrower D, Wilson L (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc Natl Acad Sci USA 90:9552–95566
19. Jun CD, Choi BM, Kim HM, Chung HT (1995) Involvement of protein kinase C during taxol-induced activation of murine peritoneal macrophages. J Immunol 154:6541–6547
20. Kaye SB (1995) Taxoids. Eur J Cancer 31:824–826
21. Lee L-F, Schuerer-Maly CC, Lofquist AK, Haften-Day C van, Ting JP, White CM, Martin BK, Haskill JS (1996) Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. Cancer Res 56:1303–1308
22. Loetscher P, Seitz M, Clark-Lewis I, Baggiolini M, Moser B (1994) Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human $CD4^+$ and $CD8^+$ T lymphocytes. FASEB J 8:1055–1060
23. Lofquist AK, Mondal K, Morris JS, Haskill JS (1995) Transcription-independent turnover of I kappa B alpha during monocyte adherence: implications for a translational component regulating I kappa B alpha/MAD-3 mRNA levels. Mol Cell Biol 15:1737–1746
24. Long BH, Fairchild CR (1994) Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. Cancer Res 54:4355–4361
25. Manthey CL, Brandes ME, Perera PY, Vogel SN (1992) Taxol increases steady-state levels of lipopolysaccharide-inducible genes and protein-tyrosine phosphorylation in murine macrophages. J Immunol 149:2459–2465
26. Manthey CL, Qureshi N, Stutz PL, Vogel SN (1993) Lipopolysaccharide antagonists block taxol-induced signaling in murine macrophages. J Exp Med 178:695–702
27. Markman M, Francis P, Rowinsky E, Hakes T, Reichman B, Jones W, Lewis JL Jr, Rubin S, Curtin J, Barakat R, et al (1994) Intraperitoneal Taxol (paclitaxel) in the management of ovarian cancer. Ann Oncol 5 [Suppl 6]:S55–58
28. Markman M, Francis P, Rowinsky E, Hoskins W (1995) Intraperitoneal paclitaxel: a possible role in the management of ovarian cancer? (Review). Semin Oncol 22[Suppl 6]:84–87
29. Moxey-Mims MM, Simms HH, Frank MM, Lin EY, Gaither TA (1991) The effects of IL-1, IL-2, and tumor necrosis factor on polymorphonuclear leukocyte Fc gamma receptor-mediated phagocytosis. IL- 2 down-regulates the effect of tumor necrosis factor. J Immunol 147:1823–1830
30. Munkarah A, Chuang L, Lotzova E, Cook K, Morris M, Wharton JT (1994) Comparative studies of taxol and taxotere on tumor growth and lymphocyte functions. Gynecol Oncol 55:211–216
31. Ogilvie AC, Hack CE, Wagstaff J, Mierlo GJ van, Erenberg AJ, Thomsen LL, Hoekman K, Rankin EM (1996) IL-1 beta does not cause neutrophil degranulation but does lead to IL-6, IL-8, and nitrite/nitrate release when used in patients with cancer. J Immunol 156:389–394
32. Ohmori Y, Strassman G, Hamilton TA (1990) cAMP differentially regulates expression of mRNA encoding IL-1 alpha and IL-1 beta in murine peritoneal macrophages. J Immunol 145:3333–3339
33. Patton LM, Saggart BS, Ahmed NK, Leff JA, Repine JE (1995) Interleukin-1 beta-induced neutrophil recruitment and acute lung injury in hamsters. Inflammation 19:23–29
34. Perera PY, Qureshi N, Vogel SN (1996) Paclitaxel (Taxol)-induced NF-kappaB translocation in murine macrophages. Infect Immun 64:878–884
35. Pluznik DH, Lee NS, Sawada T (1994) Taxol induces the hematopoietic growth factor granulocyte-macrophage colony-stimulating factor in murine B-cells by stabilization of granulocyte-macrophage colony-stimulating factor nuclear RNA. Cancer Res 54:4150–4154
36. Rao S, Krauss NE, Heerding JM, Swindell CS, Ringel I, Orr GA, Horwitz SB (1994) 3′-(p-azidobenzamido)taxol photolabels the N-terminal 31 amino acids of beta-tubulin. J Biol Chem 269:3132–3134
37. Rao S, Orr GA, Chaudhary AG, Kingston DG, Horwitz SB (1995) Characterization of the taxol binding site on the microtubule. 2-(*m*-Azidobenzoyl)taxol photolabels a peptide (amino acids 217–231) of beta-tubulin. J Biol Chem 270:20235–20238
38. Seidman AD (1994) Single-agent use of Taxol (paclitaxel) in breast cancer (review). Ann Oncol 5 [Suppl 6]:S17–22
39. Skerka C, Irving SG, Bialonski A, Zipfel PF (1993) Cell type specific expression of members of the IL-8/NAP-1 gene family. Cytokine 5:112–116

40. Strieter RM, Polverini PJ, Arenberg DA, Walz A, Opdenakker G, Van Damme J, Kunkel SL (1995) Role of C-X-C chemokines as regulators of angiogenesis in lung cancer (review). J Leukoc Biol 57:752–762
41. Su N, Ojeifo JO, MacPherson A, Zwiebel JA (1994) Breast cancer gene therapy: transgenic immunotherapy (review). Breast Cancer Res Treat 31:349–356
42. Towata T, Hayashi N, Katayama K, Takehara T, Miyoshi E, Kawanishi Y, Ueda K, Kasahara A, Fusamoto H, Kamada T (1993) Effects of cytokines on HLA class I antigen expression on Huh6 and HB611 cells. Gastroenterol Jpn 28:242–248
43. Ulmer AJ, Flad HD (1979) Discontinuous density gradient separation of human mononuclear leucocytes using Percoll as gradient medium. J Immunol Methods 30:1–10
44. Van Meir E, Ceska M, Effenberger F, Walz A, Grouzmann E, Desbaillets I, Frei K, Fontana A, Tribolet N de (1992) Interleukin-8 is produced in neoplastic and infectious diseases of the human central nervous system. Cancer Res 52:4297–4305
45. Wang X, Feuerstein GZ, Gu JL, Lysko PG, Yue TL (1995) Interleukin-1 beta induces expression of adhesion molecules in human vascular smooth muscle cells and enhances adhesion of leukocytes to smooth muscle cells. Atherosclerosis 115:89–98
46. Yagisawa M, Yuo A, Kitagawa S, Yazaki Y, Togawa A, Takaku F (1995) Stimulation and priming of human neutrophils by IL-1 alpha and IL-1 beta: complete inhibition by IL-1 receptor antagonist and no interaction with other cytokines. Exp Hematol 23:603–608