Effects of Taxol on TNF-α and IL-6 Production by Human Peripheral Blood Cells


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INTRODUCTION

Taxol, a new drug isolated from the bark of the Pacific yew tree, is being used in treating patients with breast, ovary, lung, neck, and brain cancer. The therapeutic mechanisms of taxol are not yet completely known. Taxol binds to β-tubulin, stabilizes microtubules against depolymerization, and interferes with microtubules during the cell divisions, blocking the cell through metaphase. Probably, microtubules are not the only targets of this drug.

On murine macrophages, taxol appears to have an action similar to that of bacterial lipopolysaccharide (LPS). There is some evidence that the drug binds to receptors proximal to those of LPS. LPS activates antitumor mechanisms such as biosynthesis of tumor necrosis factor alpha (TNF-α) and direct macrophage tumoricidal activity. Thus, the antitumor activities of taxol may be in part related to its ability to stimulate macrophages. The objective of this study was to determine if cells from the peripheral blood of healthy donors or tumor patients can be stimulated with taxol to produce TNF-α or IL-6 directly or in the presence of ‘‘priming’’ signals, such as those provided by interferon-gamma (IFN-γ).

METHODS

Patients

Venous blood samples from patients affected by breast cancer or age-matched healthy donors were examined. Breast tumors were diagnosed as “infiltrating ductal carcinoma” using the breast cancer classification of the Union Internationale Contre le Cancer (UICC).9

Reagents

LPS from Salmonella typhimurium, taxol, and human IFN type γ (1 × 10⁶ IRU/mg) were purchased from Sigma Chimica (Milan, Italy).
Venous Blood Cultures

Blood was diluted with an equal volume of RPMI 1640 supplemented with 100 IU of penicillin and 100 μg/mL of streptomycin. Cultures were incubated in 1-mL volumes in 24-well plates for 24 h at 37 °C in a 5% CO₂ atmosphere with or without stimuli. These were taxol (5, 10, or 20 μM), LPS (0.1 μg/mL), and/or IFN-γ (5 IU/mL). The cultures were then centrifuged and supernatants were stored at −20 °C until assayed for cytokines.

Cytokine Assays

TNF-α was measured in plasma samples by cytotoxicity in WEHI 164 clone 13 cells kindly provided by T. Espevik (Trondheim, Norway), as previously described. Briefly, WEHI cells were cultured in microtiter plates for 20 h, the culture medium was removed, and diluted samples or standards were added in duplicate in the presence of actinomycin D (1 μg/mL). Eight serial twofold dilutions (from 1:8 to 1:1.024) were tested for each plasma sample. After a 24-h incubation, the cells were stained with crystal violet and lysed to assess cytotoxicity. TNF-α activity was expressed in units per milliliter, with 1 U being defined as the amount of TNF-α causing 50% lysis of WEHI cells. The assay was calibrated by using human recombinant TNF-α (Genzyme, Cinisello Balsamo, Italy) as a standard. TNF-α activity in selected plasma samples was totally inhibited by a

![FIGURE 1. TNF-α release from whole blood cultures of healthy donors and tumor patients stimulated with taxol. Columns and bars represent means ± standard deviations of duplicate determinations of samples from six individuals per group.](image-url)
RESULTS AND DISCUSSION

Supernatants from unstimulated blood cultures did not contain measurable amounts of TNF-α or IL-6 (not shown). FIGURE 1 shows that taxol alone at three different doses (from 5 to 20 μM) was not able to increase TNF-α release over baseline levels in cultures from tumor patients or normal controls. In contrast, LPS induced considerable secretion of TNF-α in cultures from both groups. Differences in TNF-α release were not significantly different in patients and controls. Because IFN-γ was shown to considerably increase cytokine production in murine macrophages stimulated with taxol, the effects of IFN-γ were also studied. Results showed that IFN-γ, at a dose of 5 IU/mL, did not induce, alone or in combination with 20 μM taxol, significant TNF-α release from blood cultures (data not shown). Further experiments, however, are needed to assess if IFN-γ can prime isolated human monocytes to respond to taxol stimulation, as shown with murine macrophages. FIGURE 1 also shows that taxol did not have additional or synergistic effects with LPS in inducing TNF-α release.

FIGURE 2 displays IL-6 levels measured in the same samples shown in FIGURE
1. Results were similar to those observed with TNF-α in that taxol did not induce any effect, either alone or in the presence of LPS. Although taxol was shown to stimulate murine macrophages with mechanisms similar to those of LPS, very little data are available concerning human phagocytes. Preliminary results of Allen et al. also indicate that taxol may not induce cytokine expression by human monocytes. Whole blood cultures are a sensitive and convenient method to assess cytokine production by human cells. Our data show that taxol has little effect on TNF-α or IL-6 release in whole blood cultures from cancer patients or healthy controls. Further studies, however, are needed to assess if taxol, in conjunction with IFN-γ or other costimulating agents, can increase the tumoricidal activity of human macrophages.

REFERENCES