Inhibition of Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin-1 Beta (IL-1β) Secretion but Not IL-6 from Activated Human Peripheral Blood Monocytes by a New Synthetic Demethylpodophyllotoxin Derivative

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A newly synthesized demethylpodophyllotoxin derivative, 4-O-butanoyl-4'-demethylpodophyllotoxin (BDPT) or BN58705, has recently been shown to exert a potent cytotoxic activity in vitro against a variety of drug-resistant human tumor cell lines. The effect of this agent on effector cells of the immune system, however, has not been examined. The present study investigated the effect of BDPT on the response of activated human peripheral blood-derived monocytes (PBM) to secrete cytokines. Activation of PBM overnight with LPS, IFN-γ, or PMA resulted in secretion into the supernatant of TNF-α, IL-1β, IL-6, and IL-8 as assessed by ELISA. The addition of BDPT to the stimulated cultures resulted in significant inhibition of TNF-α and IL-1β secretion, whereas the secretion of IL-6 and IL-8 was not affected. The selective inhibition of TNF-α and IL-1β secretion by BDPT-treated PBM was observed with all three stimuli tested. The inhibitory effect mediated by BDPT was concentration dependent and was optimal at 6–20 μM. Time kinetic analysis indicated that the inhibition of secretion was rapid and detected as soon as 2 hr following stimulation of the PBM and lasted for as long as 24 hr. A comparison was made between BDPT and pentoxifylline, a xanthine-derived phosphodiesterase inhibitor that was reported to inhibit TNF-α and IL-1β secretion by PBM. Both BDPT and PTX showed similar time kinetics and patterns of inhibition. However, the concentration used by BDPT to achieve optimal inhibition of secretion was 10- to 20-fold less than that needed by PTX. The selective inhibition of TNF-α secretion by BDPT and PTX was corroborated by inhibition of TNF-α mRNA but not IL-6 mRNA as assessed by RT-PCR analysis. These studies demonstrate that the antitumor cytotoxic compound BDPT is also an immunomodulatory agent that can inhibit selectively TNF-α and IL-1β secretion by PBM. Further, the low toxicity and low concentrations of BDPT needed for optimal inhibition suggest that BDPT may have potential in its therapeutic application in diseases that are mediated by TNF-α and IL-1 like septic shock, inflammatory responses, and infections.

KEY WORDS: Demethylpodophyllotoxin derivative; tumor necrosis factor; septic shock; macrophages.

INTRODUCTION

Plant alkaloids are naturally occurring nitrogenous bases. The cytotoxic alkaloid derivatives of the pink periwinkle vinca alkaloids, such as vincristine and vinblastine, arrest cell division during mitosis through their ability to bind to the mitotic spindle (1). The active principle podophyllotoxin, derived from the roots and rhizomes of the mayapple or mandrake plant, was also found to be an antimitotic agent that binds to tubulin at a site distinct from that occupied by the vinca alkaloids (2). Thus, several podophyllotoxin derivatives were synthesized and were tested in many systems to determine whether there existed a relationship between their chemical structure and their antineoplastic activity.

A recent study examined the cytotoxic activity of a new demethylpodophyllotoxin derivative, 4-O-butanoyl-4'-demethylpodophyllotoxin, BN58705 or...
BDPT. The findings demonstrated that BDPT was cytotoxic against a variety of human tumor cell lines of different sensitivity to conventional chemotherapeutic drugs and could overcome the resistance of tumor cells to drugs or toxins. Further, the study demonstrated that BDPT was not very toxic following its administration in vivo in mice (3).

Many cytotoxic agents have been shown to exert various immudulatory activities in vivo and can either be immunostimulatory and contribute to the host defense mechanism or exert immunosuppressive activities that are detrimental to the host. Clearly a cytotoxic agent that can exert simultaneously both an immunostimulatory activity and an antitumor activity is very advantageous in cancer therapy. The present study initiated an investigation on the effect of BDPT on certain functions of human peripheral blood-derived monocytes (PBM). The monocytes play a central role in the immune system by acting as phagocytes, accessory antigen presenting cells, the secretion of various immunoregulatory cytokines, and their participation in inflammatory responses (4). In this study, we focused on the ability of activated human monocytes to secrete in vitro selected cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, and IL-8 and evaluated whether BDPT can affect the secretion of these cytokines. Further, we compared BDPT with pentoxifylline (PTX), a xanthine-derived phosphodisterase inhibitor, that has been shown to have many activities including the inhibition of TNF-α secretion by monocytes (5, 6).

MATERIALS AND METHODS

Reagents

BN58705 or BDPT was provided by Institut Henri Beaufour, France. It was reconstituted in DMSO at a concentration of 20 mM and was stored at 4°C. At the time of the assay, BDPT from the stock was diluted at the desired concentrations into RPMI-1640 medium supplemented with 5% bovine calf serum (BCS), nonessential amino acids (0.1 mM), sodium pyruvate (0.11 g/L), t-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml). This medium is considered complete medium in the present studies. The reagents in the complete medium were purchased from GIBCO (Grand Island, NY). Purified human rTNF-α at a specific activity of 76.6 × 10^6 U/mg and recombinant human interferon-γ (rIFN-γ) at a specific activity of 2.55 × 10^7 U/mg were provided by Genentech (San Francisco, CA). The recombinant IL-1β and IL-6 were obtained from Petrotech, NJ. Pentoxifylline was purchased from Sigma, dissolved in PBS at a concentration of 90 mM, and stored at 4°C. LPS and PMA were purchased from Sigma (St. Louis, MO).

Preparation of PBM and Lymphocytes

Peripheral blood mononuclear cells were isolated by Ficoll–hypaque density-gradient centrifugation. These cells were then allowed to adhere to plastic for 1 hr at 37°C in the presence of 5% BCS. The nonadherent lymphocytes were pipetted out, and washed twice with PBS before use. The monolayers were washed three times with PBS, the washing buffer was drained, and the side of the plate was stroked against a pad of towel papers, allowing the adherent cells to be detached by vibration. The adherent cells were then washed once, resuspended in complete medium, and adjusted to the desired cell concentration according to different protocols. The adherent cells prepared by this procedure were primarily monocytes (>90%) as determined by esterase staining and flow cytometry using markers for monocytes, T cells, B cells, and NK cells.

Stimulation of Cytokine Production by Monocytes and Lymphocytes

For studies examining the effect of BDPT on cytokine production, the PBM were incubated for 18 hr in polypropylene test tubes at 2 × 10^6 cells/ml in complete medium in the absence or presence of BDPT, PTX, and stimuli such as LPS, IFN-γ, and PMA. In most experiments, the PBM were allowed to be exposed to these agents for 18 hr. At the end of the incubation period, the culture supernatants were harvested, and after being clarified by centrifugation, they were aliquoted and frozen at −80°C until further use. The stimulation of cytokine production by lymphocytes was done following activation with Con A and IL-2.