Regulation of Tumor Necrosis Factor-α Production by a Fungal Metabolite, PR 1388

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Tumor necrosis factor-α (TNF-α) is one of the principal mediators of the inflammatory response in mammals[1]. In addition to its well-known role in acute septic shock, it has been implicated in the pathogenesis of chronic processes such as autoimmunity, graft-versus-host disease, rheumatoid arthritis, Crohn's disease, and the cachexia accompanying cancer and acquired immunodeficiency syndrome[2~7]. Therapies such as neutralizing antibodies to TNF-α and chimeric soluble TNF-α receptors have demonstrated efficacy against some of these conditions in clinical trials[8,9]. However, these protein-based therapies are unlikely to be feasible for results of development of immunogenicity, lack of oral availability and the high cost of production. Low molecular weight inhibitors of TNF-α production such as TNF-α converting enzyme (TACE) inhibitors have been developed, while those inhibitors have adverse side effects and a narrow therapeutic index due to poor bioavailability and low selectivity[10]. Accordingly, there is a need for new types of TNF-α inhibitors.

In the course of our screening program for cytokine production inhibitors, we have discovered a series of diterpenes from a fungus, *Oidiodendron griseum*[11]. Of those, a major metabolite, PR 1388 (**I**, Fig. 1) was chosen for further investigation on regulation of TNF-α production by the diterpenes. In this study, we show the effect of **I** on TNF-α production in lipopolysaccharide (LPS)-stimulated human whole blood at various concentrations and the oral efficacy on TNF-α production in LPS-challenged mice.

Materials and Methods

Isolation of PR 1388 (I)

Compound **I** was isolated from the fermentation broth of a fungus, *Oidiodendron griseum* CL37215 as described in the previous paper[11].

TNF-α Production and Leucine Uptake Assays

These assays were performed according to the methods as described previously[11].

LPS-induced Shock Model

Male BALB/C mice (6 weeks old of age) were purchased from Japan SLC (Hamamatsu, Japan). They were housed in a temperature- and light-controlled room with free access to laboratory rodent chow and water. Compound **I** dissolved in 0.1% methyl cellulose was given orally 1 hour later prior to i.v. injection with 8 mg/kg of LPS concomitantly with 0.5 mg/kg of propranolol. One hour later, blood was taken from facial vein using heparinized capillary and then transferred to sterile microcentrifuge tubes on ice. The blood samples were centrifuged at 10,000 rpm for 5 minutes at 4°C. Plasma samples were collected and stored at -30°C until subjected to TNF-α determination. Plasma TNF-α activity was measured using the L929 cytotoxicity bioassay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)[12]. One milligram of recombinant murine TNF-α corresponded to 3×10^9 laboratory units.

Results and Discussion

A fungal diterpene, **I**, was found to inhibit LPS-stimulated TNF-α production in human blood dose-

Fig. 1. Structure of PR 1388 (**I**).

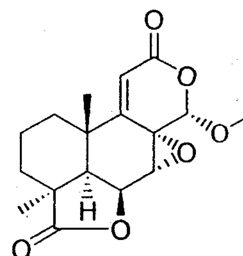

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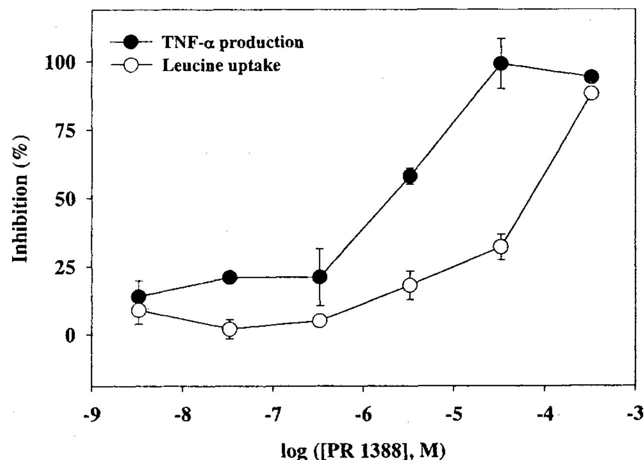
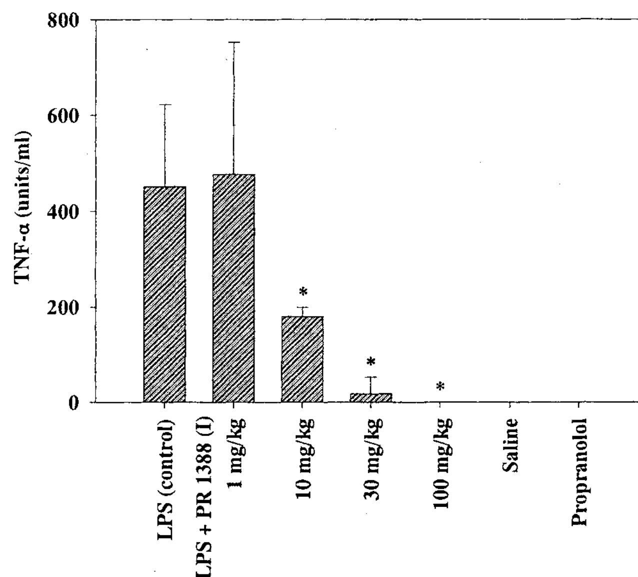
Fig. 2. Effect of PR 1388 (I) on TNF-α production and leucine uptake in human whole blood.



Data are presented as mean ± S.E.M. from three independent experiments.

Fig. 3. Effect of PR 1388 (I) on TNF-α production in LPS-challenged mice.



Data are presented as means ± S.E.M. of 5 observations.
* p<0.005, ANOVA test.

dependently (Fig. 2). The IC_{50} value of **I** was 4.7 μM. The inhibitory activity of **I** for leucine uptake was approximately 20-fold lower than that for TNF-α production.

We examined the effect of **I** on plasma TNF-α production in LPS-challenged mice (Fig. 3). The plasma TNF-α concentration 1 hour after the injection of LPS was 451.1 units/ml in LPS-challenged control mice. The plasma TNF-α production in saline or propranolol-injected mice was under detection level. Oral treatment with **I** at doses of 10, 30 and 100 mg/kg significantly attenuated the plasma TNF-α levels by 60, 96 and 100%, respectively. The ED_{50} value was calculated to be 8 mg/kg.

It has been reported that phosphodiesterase IV inhibitors significantly suppress TNF-α production due to a decrease in gene transcription[10] and that matrix metalloproteinase inhibitors block the conversion of pro-TNF-α to its secreted 17 kDa form[13]. In addition, macrophage activation by LPS results in NF-κB-dependent activation of TNF-α gene transcription, derepression of TNF-α mRNA translation, and secretion of TNF-α protein. Interleukin-4 and -13 down-regulate TNF-α mRNA translation in LPS-stimulated mouse macrophages[14]. These observations indicate that the production of TNF-α is regulated in various biosynthesis steps and there are potential target molecules regulating TNF-α production. Therefore, **I**, a new type of TNF-α production inhibitor, is thought to be a useful tool to determine the mechanism of TNF-α production. Further studies regarding the mode of action of diterpene derivatives including **I** are now in progress.

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