Structural significance of the acyl group at the C-10 position and the A ring of the taxane core of paclitaxel for inducing nitric oxide and tumor necrosis factor production by murine macrophages

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Abstract The antitumor agent, paclitaxel (Taxol®), mimics the actions of lipopolysaccharide (LPS) on murine macrophages (Mϕ). Various synthetic analogs of paclitaxel were examined for their potencies to induce nitric oxide (NO) and tumor necrosis factor (TNF) production by murine peritoneal Mϕ, and by human peripheral blood cells. The benzoyl group at C-2, the hydroxy group at C-7 and the acetyl group at C-10 were found to be critically important sites to activate murine Mϕ. Nor-seco-taxoid analogs lacking the A ring of the taxane core of paclitaxel were inactive, but inhibit paclitaxel- or LPS-induced NO production. All the compounds tested did not induce TNF production by human blood cells.

Key words: Lipopolysaccharide; Paclitaxel; Macrophage; Lipopolysaccharide low responder; Nitric oxide; Tumor necrosis factor

1. Introduction

Paclitaxel (Taxol®), a complex diterpene isolated from the stem bark of *Taxus brevifolia*, has antiproliferative activity against various cultured cells, antitumor activity in vivo [1–4], and is currently used as a leading drug for cancer chemotherapy. The activity is related to its ability to bind β-tubulin, to promote microtubule assembly, and to stabilize microtubules by bundle formation [5]. Paclitaxel can mimic certain effects of bacterial lipopolysaccharide (LPS) on murine peritoneal macrophages (Mϕ) [6] although it has no chemical structural similarity to LPS. Mϕ stimulated with LPS produce various active mediators, including nitric oxide (NO) and tumor necrosis factor (TNF), and they cause endotoxic shock [7,8]. Peritoneal Mϕ derived from LPS-responsive C3H/HeN mice secrete the mediators when stimulated with paclitaxel or LPS, whereas the LPS-hyporesponsive C3H/HeJ Mϕ [9,10] cannot secrete these mediators in response to either LPS or paclitaxel [6,9–13]. Genetic analysis of recombinant inbred mice revealed a close linkage between the mutant *LPS*^{d} allele and the Mϕ inability in response to paclitaxel as well as LPS [6]. These data have provided strong evidence that paclitaxel shares an intracellular signal transduction pathway with LPS.

We [14,15] and Burkhart et al. [16] have recently reported structure–activity relationships of a set of synthetic paclitaxel analogs (taxoids) for their potencies to induce NO and TNF production by murine C3H/HeN and C3H/HeJ Mϕ and to inhibit the growth of Mϕ-like cells. The data indicate that some parts of the structure in taxoids are related with their abilities to activate Mϕ, and the presence of an aromatic group in the *N*-acylamino group at the C-3′ position is the most important structure to activate C3H/HeN Mϕ [15]. In addition, we also found that the *p*-position of the aromatic group at the C-3′ position has a significant effect on the activity [14]. In the present study, synthetic analogs of paclitaxel modification at C-2, C-7 and C-10 positions and synthetic analogs of nor-seco-taxoids lacking the A ring of the taxane core of paclitaxel were examined for their potencies to induce NO and TNF production by peritoneal Mϕ of LPS-responsive C3H/HeN and -hyporesponsive C3H/HeJ mice, and by human peripheral blood cells.

2. Materials and methods

2.1. Paclitaxel and taxoids

Paclitaxel was obtained from Sigma Chemical Co.; St. Louis, MO, USA. Taxoids (**1**–**23**) and nor-seco-taxoids (**24** and **25**) were synthesized by means of the β-Lactam Synthon Method [17–20]. The analogs tested and their structures are given in Table 1A,B. Paclitaxel and taxoids were stored as a 10 mM stock solution in dimethylsulfoxide (DMSO), and dissolved as reported previously [15].

2.2. Reagents

The Re-chemotype LPS from *Salmonella minnesota* R595 LPS was kindly provided by Dr. K. Hisatsune, Josai University, Sakado, Saitama, Japan. The Ra-chemotype LPS from *S. minnesota* R60 LPS was purchased from Sigma Chemical Co., and it was used only in the experiments using C3H/HeJ Mϕ, because C3H/HeJ Mϕ could hardly respond to Re-chemotype LPS [21]. Murine recombinant interferon γ (IFNγ) was donated from Shionogi Pharmaceutical Co., Osaka, Japan.

2.3. Mice

C3H/HeN and C3H/HeJ mice were bred and maintained in the Animal Facility of the Jichi Medical School. Female mice were used at 10–15 weeks of age.

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Abbreviations: IFNγ, interferon γ; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; Mϕ, macrophages; NO, nitric oxide; TNF, tumor necrosis factor

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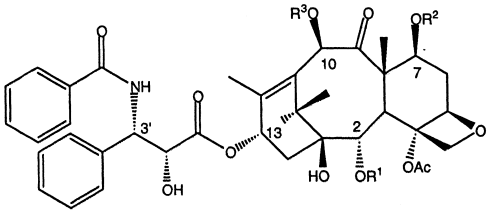
2.4. Mϕ stimulation

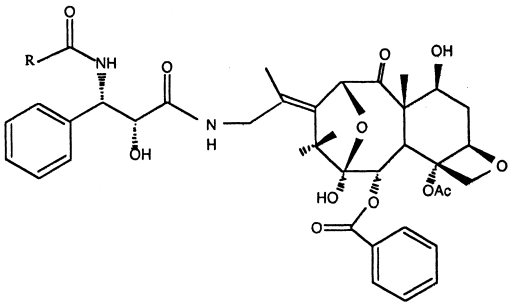
Thioglycolate-elicited murine peritoneal Mϕ, a murine Mϕ-like cell line, J774.1, and its mutant cell line, J7.DEF.3 [22] were plated in 96-well plates and stimulated with taxoids or LPS as reported previously [15]. The J7.DEF.3 cell line is defective in expression of CD14 antigen [23], a receptor for the complex of LPS and LPS-binding proteins (reviewed in [8]). In some experiments, Mϕ were preincubated with taxoids for 2 h, and then stimulated with LPS or paclitaxel. The culture supernatants were obtained 4 h (for TNF assay) and 48 h (for NO assay) after the incubation.

Table 1A
Chemical structures of paclitaxel and taxoids



compound	R^1	R^2	R^3
paclitaxel	–CO–Ph	H	Ac
1	Ac	H	Ac
2	–COCH$_2$CH(CH$_3$)$_2$	H	Ac
3	–COCH=C(CH$_3$)$_2$	H	Ac
4		H	Ac
5		H	Ac
6	–CO–c-C$_6$H$_{11}$	H	Ac
7	–CO(CH$_2$)$_2$–Ph	H	Ac
8	–COCH=CH–Ph	H	Ac
9	–CO–Ph	Ac	Ac
10	–CO–Ph	–CO(CH$_2$)$_3$CH$_3$	Ac
11	–CO–Ph	–COCH$_2$CH(CH$_3$)$_2$	Ac
12	–CO–Ph	–COCH=C(CH$_3$)$_2$	Ac
13	–CO–Ph	–COCH=CH–Ph	Ac
14	–CO–Ph	H	–COCH$_2$CH$_3$
15	–CO–Ph	H	–CO(CH$_2$)$_2$CH$_3$
16	–CO–Ph	H	–CO(CH$_2$)$_4$CH$_3$
17	–CO–Ph	H	–CO(CH$_2$)$_{10}$CH$_3$
18	–CO–Ph	H	–CO(CH$_2$)$_{12}$CH$_3$
19	–CO–Ph	H	–CO–c-C$_6$H$_{11}$
20	–CO–Ph	H	–COCH=CHCH$_3$
21	–CO–Ph	H	–CO–Ph
22	–CO–Ph	H	–CON(CH$_3$)$_2$
23	–CO–Ph	H	–CO–N(CH$_2$CH$_2$)$_2$O

Table 1B
Chemical structures of nor-seco-taxoids



compound	R
24	–CO(CH$_3$)$_3$
25	

2.5. Stimulation of human peripheral blood samples

Fresh heparinized blood samples were obtained from healthy volunteers and were diluted four-fold with serum-free RPMI 1640 medium, plated and then taxoids or LPS were added as described previously [14]. In some experiments, the diluted samples were incubated with paclitaxel for 2 h, and then stimulated with 1 ng/ml of LPS. The culture supernatants were collected for TNF assay.

2.6. TNF and NO assays and detection of inducible NO synthase (iNOS) in Mϕ

TNF activity was determined by a functional cytotoxic assay using actinomycin D-treated L929, as described previously [13]. NO formation was measured as the stable end product nitrite (NO_2^-) in culture supernatants using the Griess reagent [13]. iNOS in the Mϕ was detected by Western blot with anti-iNOS antibody as described previously [15].

2.7. Cell growth inhibition assay

The growth of J774.1 cells or J7.DEF.3 cells were monitored by a quantitative calorimetric staining assay using a tetrazolium salt (MTT) as described previously [15].

3. Results

3.1. NO and TNF production by murine peritoneal Mϕ stimulated with paclitaxel analogs

Twenty six compounds including paclitaxel (Table 1A,B) were examined for their ability to induce NO and TNF production by the C3H/HeN and C3H/HeJ peritoneal Mϕ, and the results for C3H/HeN Mϕ are summarized in Table 2. The dose–responses by C3H/HeN Mϕ to some of the active compounds are shown in Fig. 1A,B. Taxoids **1**–**3**, **6**–**8**, **10**–**13**, **19** and **22**–**25** with the range of 1–30 μM did not show any ability to induce NO and TNF production by these Mϕ. Taxoids **5**, **18** and **20** induced weak TNF production by C3H/HeN Mϕ, however, it did not induce any detectable NO production. The other eight compounds (paclitaxel and taxoids **4**, **9**, **14**–**17** and **21**) definitely induced both NO and TNF production by C3H/HeN Mϕ. Among them, paclitaxel and taxoids **9**, **16**, **17** and **21** showed strong activity in inducing NO and TNF production. Especially, taxoid **17** was more active than paclitaxel, i.e.

the minimal inducing dose of paclitaxel was 1.9 μM, whereas that of taxoid **17** was 0.9 μM (Fig. 1). The dose–response curves for taxoid **17** shifted to about two-fold lower concentration in comparison with those for paclitaxel. The results suggest that NO and TNF inducibility by these compounds are fundamentally correlated. LPS-hyporesponsive C3H/HeJ Mϕ could not produce any detectable NO and TNF in response to the active taxoids, even in the presence of IFNγ (2 U/ml) except for taxoid **5**. Taxoid **5** showed a unique ability to induce TNF production by both C3H/HeN Mϕ (Fig. 1) and C3H/HeJ Mϕ (Fig. 2) supplemented with IFNγ, albeit with weak ability. However, taxoid **5** could not induce NO production at all.

3.2. Structural requirement of active compounds to induce NO/TNF production by Mϕ

Some special components or groups at the C-2, C-7 and C-10 positions in taxoids are required for the induction of NO/TNF production by C3H/HeN Mϕ. The benzoyl group at the C-2 position is an important structure to induce NO/TNF production by the Mϕ, i.e. replacement of the benzoyl group with an acetyl (**1**), isovaleryl (**2**), 3-methyl-2-butenoyl (**3**), cyclohexanecarbonyl (**6**), 3-phenylpropionyl (**7**), or cinnamoyl (**8**) group resulted in the substantial loss of activity and those with 3,3-dimethyl-4-pentenoyl (**4**) and 3,3-dimethylpentanoyl (**5**) groups resulted in a diminished activity. A hydroxy (paclitaxel) or acetyl group (**9**) at the C-7 position is required for the induction of NO/TNF, i.e. the introduction of a valeryl (**10**), isovarelyl (**11**), 3-methyl-2-butenoyl (**12**), or cinnamoyl (**13**) group to this position resulted in loss of the activity. The substitution of the acetyl group at the C-10 position especially affected the activity. The introduction of a dodecanoyl group (**17**) to the C-10 position resulted in a distinct gain of activity. The substitution of a hexanoyl (**16**) or benzoyl group (**21**) virtually did not affect the activity, whereas the substitution of a propanoyl (**14**), butanoyl (**15**), tetradecanoyl (**18**), cyclohexanecarbonyl (**19**), 2-butenoyl (**20**), dimetylcarbamoyl (**22**) or 4-morpholinecarbonyl (**23**) group resulted in the decrease or substantial loss of the activity. The A ring of the taxane core of paclitaxel is an important structure to induce NO/TNF production by the Mϕ, i.e. nor-seco-taxoids lacking the A ring of the taxane core (**24** and **25**) were completely inactive.

Table 2
Cell-growth inhibition and macrophage activation by taxoids

Compounds	Mouse macrophage activation C3H/HeN		Growth inhibition (IC_{50}, nM)[b]	
	TNF	NO	J774.1	J7.DEF.3
paclitaxel	+++++[a]	++++	33	30
1	−	−	>1000	>1000
2	−	−	>1000	>1000
3	−	−	394	393
4	+	+	509	508
5	+	−	508	512
6	−	−	339	448
7	−	−	>1000	>1000
8	−	−	>1000	>1000
9	+++++	++++	39	42
10	−	−	>1000	>1000
11	−	−	34	35
12	−	−	44	45
13	−	−	>1000	>1000
14	+++	++	39	37
15	++	+	43	36
16	++++++	++++	35	32
17	++++++	+++++	622	443
18	+	−	>1000	>1000
19	−	−	41	35
20	+++	−	15	28
21	+++++	+++	26	32
22	−	−	27	30
23	−	−	41	45
24	−	−	>1000	>1000
25	−	−	>1000	>1000

[a]Symbols, ++++++ to ++, indicate the minimal concentration of a taxoid to induce significant amounts of NO (>4 μM) or TNF (>20 U/ml); ++++++ <0.9 μM, +++++ <1.9 μM, ++++ <3.8 μM, +++ <7.5 μM, ++ <15 μM; + indicates the concentration of a taxoid to induce them was more than 30 μM or 1–4 μM NO or 10–20 U/ml TNF was induced; − indicates that no significant NO (<1 μM) or TNF (<10 U/ml) was induced. The data on NO and TNF production were induced from three and two independent experiments, respectively. Data for paclitaxel are shown for comparison.

[b]The concentration of a taxoid which inhibited 50% (IC_{50}) of the growth of J774.1 cells and J7.DEF.3 cells, after 72 h incubation with the taxoid. The data represent the mean values of the three independent experiments. Data for paclitaxel are shown for comparison.

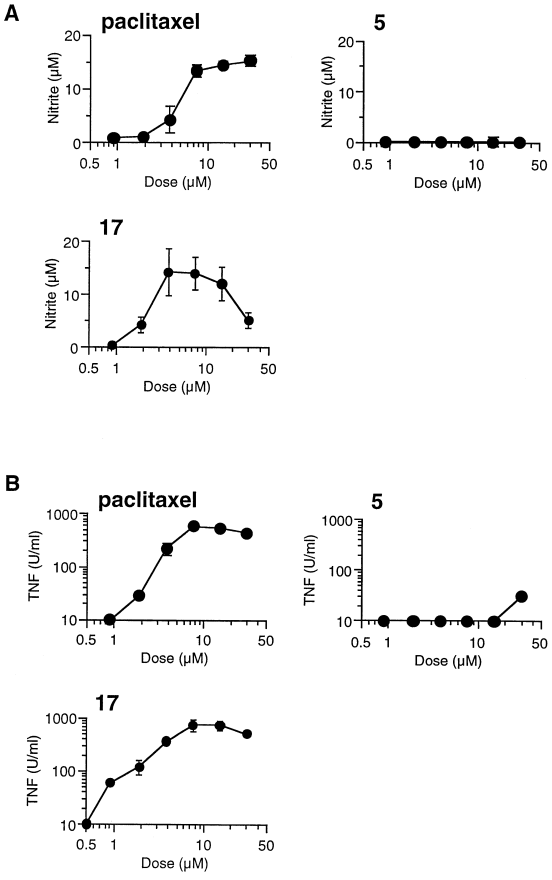


Fig. 1. Taxoid-induced NO and TNF production by C3H/HeN Mϕ. C3H/HeN Mϕ were incubated with various doses of paclitaxel and taxoids **5** and **17** for 48 h for the NO assay or 4 h for the TNF assay. A: NO production was determined by measuring nitrite accumulation in the supernatants in triplicate with Griess reagent. Each point represents the mean ± standard error of the mean of three or four experiments. B: TNF activity in the supernatants was determined by the cytotoxic assay in triplicate using L929 cells. The data are from one of the two experiments with similar results.

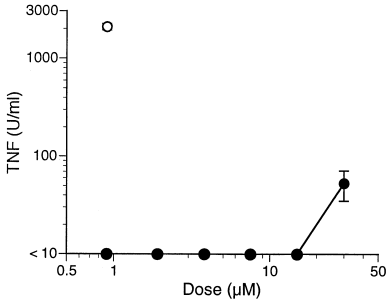


Fig. 2. LPS- or taxoids-induced TNF production by C3H/HeJ Mϕ. Mϕ from C3H/HeJ were incubated with 10 ng/ml LPS (open circle) or taxoid **5** (closed circle) for 4 h. TNF activity in the supernatants was determined by the cytotoxic assay in triplicate using L929 cells. The data shown are from one of the three experiments with similar results.

3.3. *TNF production by human peripheral blood cells stimulated by LPS and paclitaxel analogs*

To confirm the effect on human cells, we assessed TNF production by human peripheral blood cell cultures in response to LPS and taxoids. As previously reported [13], LPS induced significant TNF production by human peripheral blood cells with optimum response at 18 h, and the response was dose-dependent with a minimal inducing dose of 0.1 ng/ml. However, neither taxoids nor nor-seco-taxoids tested with the range of 1–30 μM did show any ability to induce NO/TNF production (data not shown).

3.4. *Effects of pretreatment with taxoids on TNF/NO production by murine peritoneal Mϕ and human blood cells in response to paclitaxel or LPS*

To determine whether the taxoids modulate the LPS- or paclitaxel-induced TNF production, C3H/HeN Mϕ and human blood samples were preincubated with the taxoids (15 or 30 μM) for 2 h, and then stimulated with 15 μM paclitaxel or 1 ng/ml LPS. Taxoids **1**–**3**, **6**–**8**, **10**–**13**, **19**, **22** and **23**, which were inactive to the Mϕ, did not affect or barely suppressed paclitaxel- or LPS-induced TNF/NO production (data not shown). The same treatment with inactive nor-seco-taxoids **24** and **25** did not affect paclitaxel- or LPS-induced TNF production (data not shown), but effectively inhibited NO production induced either by paclitaxel or by LPS in a dose-dependent manner (Fig. 3). Pretreatment of human blood cells with all 25 compounds tested including nor-seco-taxoids following paclitaxel- or LPS-stimulation did not induce any detectable TNF production (data not shown).

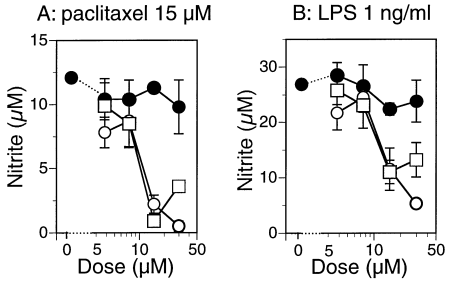


Fig. 3. Effects of pretreatment with nor-seco-taxoids **24** and **25** on LPS- or paclitaxel-induced NO production by C3H/HeN Mϕ. C3H/HeN Mϕ were preincubated with various doses (indicated on the abscissa) of **24** (open circle) and **25** (open square) or with DMSO (closed circle) equivalent to its concentration contained in these nor-seco-taxoid solutions for 2 h, and then cultured in the presence of 15 μM paclitaxel (A) or 1 ng/ml LPS (B) for 48 h. NO production was determined by measuring nitrite accumulation in the supernatants with Griess reagent in triplicate. Each point represents the mean standard error of the mean of three or four experiments.

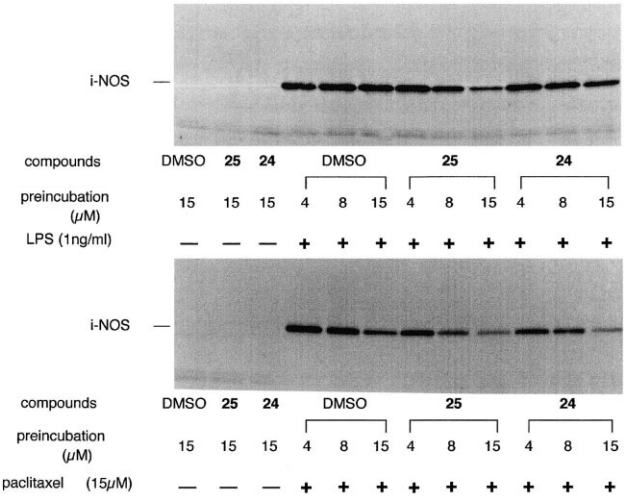


Fig. 4. Nor-seco-taxoids inhibit iNOS production. C3H/He Mϕ were preincubated with 4, 8 and 15 μM of **24** and **25** or with DMSO equivalent to its concentration contained in these nor-seco-taxoid solutions for 2 h, and then stimulated with 15 μM paclitaxel (lower panel) or 1 ng/ml LPS (upper panel) for 16 h. Expression of iNOS protein in Mϕ was detected by Western blot analysis with rabbit anti-iNOS antibody. The iNOS protein expression was quantified by Quantity One (Huntington Station, NY, USA). The data are from one of the four experiments with similar results.

3.5. *Effects of pretreatment with nor-seco-taxoid on induction of iNOS expression in murine peritoneal Mϕ in response to paclitaxel or LPS*

Since the pretreatment of C3H/HeN Mϕ with nor-seco-taxoid compound **24** or **25** suppressed paclitaxel- or LPS-induced NO production, we examined whether or not the suppression was seen on iNOS expression level. As Fig. 4 shows, the suppression was indeed seen at the level of iNOS expression.

3.6. *Growth inhibitory effect of taxoids on murine Mϕ-like cell lines*

Cell growth inhibitory potencies of the taxoids were examined against murine Mϕ-like CD14-positive J774.1 cells and CD14-negative J7.DEF.3 mutant cells, and the results are summarized in Table 2. Paclitaxel and taxoids **9**, **11**, **12**, **14**–**16** and **19**–**23** inhibited the growth of both cell lines effectively. Taxoids **3**–**6** and **17** showed lower activity. Taxoids **1**, **2**, **7**, **8**, **10**, **13** and **18** as well as nor-seco-taxoids **24** and **25** were inactive.

3.7. *No correlation between the cell growth inhibitory activity and NO/TNF inducibility*

As shown in Table 2, any correlation is hardly seen between anti-proliferative activities of paclitaxel and taxoids against murine Mϕ-like J774.1 as well as J7.DEF.3 cells and their ability to induce NO and TNF production by the C3H/HeN Mϕ. Paclitaxel and taxoids **9**, **16** and **21** showed strong potencies to induce NO/TNF production and to inhibit the cell growth. On the contrary, taxoids **11**, **12**, **19**, **22** and **23** did not

show any meaningful activity in inducing NO/TNF production, but retained the ability to inhibit the cell growth. Whereas taxoid **17** showed strong potency to induce NO/TNF production, it showed no potency to inhibit cell growth. These findings indicate that the potency of taxoids to induce NO and TNF production by the murine Mϕ is not correlated to their growth-inhibitory effect on the murine cells.

4. Discussion

We examined the effects of paclitaxel, its 23 analogs with modification of the benzoyl groups at the C-2 position, the hydroxy group at the C-7 position and the acetyl group at the C-10 position (R^1, R^2 and R^3 in Table 1A,B, respectively), and two nor-seco-taxoids on the in vitro inducibility of NO and TNF production by LPS-responsive C3H/HeN and LPS-hyporesponsive C3H/HeJ Mϕ as well as NO production by the human blood cells. None of compounds except taxoid **5** induced the TNF production by C3H/HeJ Mϕ (Fig. 2), even when the Mϕ were primed with IFNγ. On the other hand, C3H/HeN Mϕ produced both NO and TNF in response to some of the taxoids (Table 2), indicating that the LPS-mimicking activity, like paclitaxel that can activate C3H/HeN Mϕ but not C3H/HeJ Mϕ, is retained in these compounds. Also it is clearly demonstrated that the pretreatment of the Mϕ with nor-seco-taxoids (**24** and **25**), which are inactive to induce TNF and NO production (Table 2), can inhibit NO production (Fig. 3) but not TNF production induced by paclitaxel or LPS (data not shown).

Paclitaxel mimics LPS for its certain effects, including its inducibility of NO and TNF production by Mϕ of LPS-responsive mouse strain, but not Mϕ of genetically LPS-hyporesponsive strain [6,13,24,25]. Some taxoids (**4**, **9**, **14**–**18**, **20** and **21**) can also mimic the LPS effects on genetic basis, i.e. those compounds activate LPS-responsive C3H/HeN Mϕ (Table 2), but not LPS-hyporesponsive C3H/HeJ Mϕ. Taxoid **17** which has a dodecanoyl group at the C-10 position among the taxoids tested shows the strongest ability to induce NO/TNF production by C3H/HeN Mϕ (Table 2 and Fig. 1). Taxoid **16** with a hexanoyl group at the C-10 position also shows strong activity. However, taxoids **14** and **15**, which have propionyl and butyryl groups at the same position, respectively, possess weak activity. Furthermore, taxoid **18** with a tetradecanoyl group at the same position almost lost activity. These findings suggest that the acyl substituent at the C-10 position is very important for the activity and the length of the acyl chain seems to determine the strength of the activity. It is well documented that, in the lipid A portion of LPS, an adequate length of acyl chain is essential to provoke the endotoxic activity [8]. Accordingly, it is possible to assume that the acyl group at the C-10 position of paclitaxel plays a role similar to that of the acyl group of lipid A.

LPS as well as paclitaxel bind to microtubules [26,27]. Microtubules are known to associate with many cell functions such as regulation of TNFα receptor expression on murine Mϕ [6,28], activation of membrane-bound GTPase [29], association with mitogen-activated protein kinases [30,31], LPS-induced interleukin-1 production by human monocytes [30] and LPS and paclitaxel-induced NO synthesis [22]. These functions of microtubules may be influenced by the binding of LPS or paclitaxel, and presumably of active taxoids. It has been shown that paclitaxel and its analogs inhibit the cell growth by stabilizing microtubules [20]. However, microtubules themselves do not seem to act as the common receptors for LPS and paclitaxel. In fact, our previous study [13] demonstrated that microtubules did not serve as functional receptors for either LPS or paclitaxel to induce TNF and NO production by murine Mϕ since LPS did not inhibit the binding and biological action of isotopically labeled paclitaxel to Mϕ and vice versa. Inhibition of cell growth by paclitaxel is thought to be due to its binding to microtubules [32]. Among these active taxoids, no correlation was observed between TNF/NO production and cell growth inhibition (Table 2). These findings may suggest a possibility that the receptors on Mϕ initiating TNF/NO-induction are different from those triggering cell growth inhibition. A recent study demonstrated that the mutation of C3H/HeJ mice was located in the *tlr4* gene coding toll-like receptor (TLR) 4, indicating that TLR 4 was a functional receptor for LPS [33]. TLR 4 may serve as the receptor of murine Mϕ for paclitaxel as well as its active analogs. It was reported that in human cells TLR 2 seemed to be more important than TLR 4 in response to LPS [34]. Presumably taxoids may not use TLR 2 on human peripheral blood cells since the cells do not respond to taxoids [14].

It is worth mentioning that taxoid **5**, an analog having a 3,3-dimethylpentanoyl group at the C-2 position, is active in both C3H/HeN and C3H/HeJ Mϕ (Figs. 1B and 2). We recently suggested that LPS from oral black-pigmented bacteria induces TNF production by C3H/HeJ Mϕ as well as C3H/HeN Mϕ in a manner different from that of *Salmonella* LPS [21]. Oral black-pigmented bacterial LPS and taxoid **5** may use other functional receptor(s) different from TLR 4, such as other TLR families [35].

In a previous paper [36], we reported that a pretreatment of C3H/HeN Mϕ with inactive nor-seco-taxoid **25** did not suppress subsequent paclitaxel- or LPS-induced TNF production, but suppressed NO production, indicating that taxoid **25** has the capability of conditioning Mϕ, albeit that it does not have ability to induce TNF/NO production. In the present study, we have found that the majority of non-active taxoids show little conditioning effect on paclitaxel- or LPS-induced TNF/NO production. However, the conditioning of C3H/HeN Mϕ with nor-seco-taxoids **24** and **25** apparently affects the production of NO, but not TNF, by a stimulation with either paclitaxel or LPS (Fig. 3). This result suggests that these compounds are not inactive, but have some priming effect. This effect can be ascribed to the hindrance of iNOS expression after the stimulation (Fig. 4). Further study is needed to clarify how the conditioning of murine Mϕ with nor-seco-taxoids suppresses paclitaxel- or LPS-induced NO production and why the same pretreatment of human blood cells does not affect it.

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