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Mechanisms by which Chemotherapeutic Agents Augment the Antitumor Effects of Tumor Necrosis Factor: Involvement of the Pattern Shift of Cytokines from Th2 to Th1 in Tumor Lesions

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Abstract. *Background: The antitumor effect exerted by tumor necrosis factor (TNF) is characteristic in that it causes central necrosis of the tumor mass. Viable tumor cells surrounding the tumor mass remain, however, even after most of the mass is necrotized, and these cells gradually regrow and form tumors. To overcome this, we analyzed the combined effects of chemotherapeutic agents used with TNF. Alkylating agents such as cyclophosphamide altered the antitumor effect qualitatively, leading to complete regression which TNF alone could not achieve. The mechanism, behind the enhancement of endogenous TNF production and expression of mRNA of various cytokines by the combination of chemotherapeutic agents with TNF inducer was investigated in Meth A fibrosarcoma. Methods: Seven days after the inoculation of Meth A fibrosarcoma into BALB/c mice, cyclophosphamide (CY, 100-150 mg/kg) was injected intraperitoneally, and 7 days later endogenous TNF was induced by the intradermal administration of lipopolysaccharide (LPS, 400μg/kg) or intravenous injection of ONO-4007, a synthetic lipid A derivative (30mg/kg). Results: A combination therapy of LPS or ONO-4007 with CY showed the effect of complete regression in 50-100% of tested mice, while CY, LPSp or ONO-4007 alone did not cause complete regression. The amount of endogenous TNF induced by LPSp or ONO-4007 around a tumor lesion with CY was 4-5 fold higher than that without CY. The expression of mRNA of transforming growth factor-β was suppressed by CY seven days after the injection, and expressions of mRNA of IL-1β and TNF-α were augmented by the administration of CY 1 to 3 hours after the administration of ONO-4007. Conclusion: Some chemotherapeutic agents thus appear to augment the antitumor effect of TNF around tumor lesions, leading to tumor regression through a mechanism in which the agent changes the host's immune status, especially around a tumor lesion and pattern shift of cytokines from Th2 to Th1.*

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Systemic administration of tumor necrosis factor-α (TNF-α) shows dose-limiting toxicity at a dose with no antitumor activity (1-3), yet it remains a promising antitumor cytokine because of its unique characteristics which may lead to tumor regression if it can be administered to humans at the same doses which achieve regression in mice. The maximum tolerable dose of TNF-α by systemic administration to human patients is now 8-10 μg/kg body weight, while tumor regression in mice requires a dose of approximately 400μg/kg (2,4). Various approaches have been made, including construction of a novel recombinant TNF mutein with less toxicity (5), and combination with a drug delivery system capable of administering TNF at high doses (6); these showed that a high dose of TNF could achieve a considerable antitumor effect even in tumor patients (7). Among these trials, the most dramatic results achieved using TNF in antitumor therapy are those demonstrated by isolation limb perfusion with a high dose of TNF, especially to melanoma and soft tissue sarcoma (7-9). These suggest that if we could induce a high concentration of TNF of over 0.1μg/g around a tumor lesion, it might exert a much stronger antitumor effect than can be anticipated by the administration of conventional drugs. We have already demonstrated that TNF is concentrated around tumor lesion following i.d. administration of lipopolysaccharide (LPS) (10), or endogenous and exogenous TNF (EET) therapy (11,12) all of which produce a considerable amount of endogenous TNF (approximately 0.1μg/g) around a lesion in an animal model.

It is also well known that antitumor effects by TNF characteristically cause central necrosis of the tumor, but that viable tumor cells are left surrounding the tumor mass and regrow after the therapy. If we can modify the antitumor

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effect of TNF so that it is effective even at the rim lesion of a tumor mass, it will be helpful in constructing a novel antitumor therapy with TNF. Yamazaki (13) and Tomita (14) reported that the combination therapy of mitomycin (MMC) with EET showed a more effective antitumor effect than EET therapy alone. Moreover, Eggermont *et al* demonstrated that melphalan is an essential property to obtain a complete antitumor responses by isolation limb perfusion with a high dose of TNF (7-9), and that only the combination of TNF with melphalan showed complete necrosis of the tumor both in the center and on the rim (6).

Melphalan, an alkylating chemotherapeutic drug, suppressed the production of Th2 type cytokines such as TGF-ß in large MOPC-315 tumor bearing mice (15), while it promoted the production of the Th1 type cytokines TNF (16), IL-12 and interferon-γ (17), these results demonstrated that melphalan modulated the Th1/Th2 cytokine pattern in the tumor bearing host. From these results, we assumed that some chemotherapeutic agents might alter the characteristics of TNF and lead to complete tumor regression by TNF. Elucidation of the roles and mechanisms enhancing TNF by a chemotherapeutic drug is therefore a good target not only to reevaluate the novel role of the chemotherapeutic drug in tumor therapy in the regulation of immune response, but also to establish the protocol of a new bio-chemotherapy based particularly on TNF-α. Thus, we analyzed the antitumor effect of the combination of intradermal (i.d.) administration of *Pantoea agglomeranse* LPS (LPSp), a low molecular size LPS (18), and intravenous (i.v.) administration of ONO-4007, a synthetic lipid A derivative (19,20), with cyclophosphamide (CY), focusing on the amount of TNF induced around a tumor lesion and on the change of expression pattern of Th1/Th2 cytokines.

Materials and Methods

Mice. BALB/c, C3H/He, and C57BL/6 male mice were purchased from Shizuoka Experimental Animal Farm (Shizuoka, Japan) or Seac Yoshitomi (Fukuoka, Japan). They were 6-12 weeks of age at the start of the experiments. All mice were given a standard laboratory diet and water *ad libitum.*

Tumor. Meth A fibrosarcoma and MH 134 hepatoma were passaged once a week as ascites in BALB/c and C3H/He mice, respectively. Lewis lung carcinoma was passaged in C57BL/6 mice as a solid tumor once every two weeks.

Reagents. CY was purchased from Wako Pure Chemical Industries (Osaka, Japan). Melphalan was purchased from Sigma (MO, USA). LPSp was extracted and purified from *Pantoea agglomerans,* a Gram-negative bacterium, by the hot/phenol method of Westphal in our laboratory (18,21). ONO-4007, a synthetic lipid A derivative (19,20) was kindly supplied by ONO Pharmaceutical Company (Osaka).

Therapeutic experiment. Meth A fibrosarcoma (2×10^5 cells) or MH134 hepatoma (2×10^5 cells) was inoculated i.d. into the abdomen of BALB/c or C3H/He mice, respectively. Treatment was started after the mice had been grouped according to tumor diameter on day 7 after tumor inoculation. CY (100-150 mg/kg) was administered intraperitoneally (i.p.) on that same day and endogenous TNF induction was performed on day 14 after the tumor inoculation. Endogenous TNF induction was performed with two protocols: (i) i.d. administration of LPSp (400μg/kg), and (ii) i.v. administration of ONO-4007 (30mg/kg). The tumor diameter was given as the square root value obtained by multiplying the major axis (a) by the minor axis (b) of the tumor measured every 2 to 3 days by vernier calipers using the following calculation:

tumor diameter (mm) $=\sqrt{ab}$

Induction of endogenous TNF in tumor lesion. Details of the procedure to obtain TNF-containing samples was described previously (22). Briefly, Meth A fibrosarcoma (BALB/c) bearing mice were treated by TNF inducer. Ninety minutes after injection, tumor tissues were taken, weighed, minced and homogenized. Supernatant of the homogenized sample obtained by centrifugation (7000g x 10minutes) was kept at -80°C until use. TNF was assayed by the method described previously (22,23).

Reverse transcription-polymerase chain reaction (RT-PCR). Meth A fibrosarcoma bearing BALB/c mice were administered CY (150 mg/kg) on day 7 after tumor inoculation and treated with endogenous TNF inducer (ONO-4007 30mg/kg) on day 14 after the tumor inoculation. One to 24 hours after injection of ONO-4007, tumor tissues were taken, and total RNA was extracted from them using an acid-guanidium-phenol-chloroform method. Expressions of messenger RNA of cytokines were detected by RT-PCR. Briefly, PCR was performed for 40 cycles, each consisting of 1 minute denaturation at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C. The PCR products were electrophoresed on agarose gel containing ethidium bromide. The sequence of PCR primers is shown in Table 1. Relative amount of PCR product was stratified into four grades (high; ++, medium; +, low; ±, not detected; -) referring to the intensity of agarose gel electrophoresis of the molecular marker (pSP64/HinfI digested products, 0.5 μg/lane: 1198bp ;++, 517bp; +, 396bp; +, 354bp; +, 218bp; ±, 151bp; ±).

Statistical analysis. Statistical evaluations of differences between groups were made by Student's t-test and Mann-Whitney's U test.

Results

Antitumor effects of LPSp or ONO-4007 in combination with CY against the Meth A fibrosarcoma, MH134 hepatoma and Lewis lung carcinoma model. Some alkylating agents such as CY were found to markedly synergize the antitumor effect by TNF (24,25). We also previously reported that CY was the best chemotherapeutic agent in comparison with 5 other agents (tegaful, adriamycin, actinomycin D, MMC and puromycin) which are combined with LPS to augment endogenous TNF production around tumor lesions, and that the optimal interval between CY injection and administration of LPS to obtain maximum antitumor effect in a mouse tumor model was 7 days (26). The effect of i.d. administration of LPS (400μg/kg) or i.v. administration of ONO-4007 (30mg/kg) combined with CY on mice bearing Meth A, MH134 or Lewis lung carcinoma is shown in Table II. Complete tumor regression was not observed in the group treated with LPS, ONO-4007 or CY. In contrast, the group treated with CY on day 15 after tumor inoculation followed by LPS or ONO-4007 administration showed a marked inhibition of tumor growth with 50 to 100% of complete regression in the mice.

Table I. *Oligonucleotide primers of 11 target genes used for PCR experiments.*

Gene	Sense primer 5'	3'	Antisense primer 5'	3'	Product size(bp)
ß-actin	CCAACCGTGAAAAGATGACC		CAGGAGGAGCAATGATCTTG		660
IL-1ß	GGCTGCTTCCAAACCTTTGA		GAAGACACGGATTCCATGGT		710
TNF-α	AGCACAGAAAGCATGATCCG		GGAGTAGACAAGGTACAACC		414
IL-12(p40)	ACTGGACCAAAGGGACTATG		AATAGCGATCCTGAGCTTGC		421
IL-6	GCTATGAAGTTCCTCTCTGC		CTAGGTTTGCCGAGTAGATC		539
IL-10	CTGGCATGAGGATCAGCAGG		CACCTGCTCCACTGCCTTGC		386
TGF-ß	CCTGAGTGGCTGTCTTTTGA		GCGCACAATCATGTTGGACA		578
Fas	GTGTGAACATGGAACCCTTG		TTCATTTCCAGTGTCTGGGG		529
Fas-L	CTGGAATGGGAAGACACATA		AAAGGTCTTAGATTCCTCAA		346
TNF-RI	CCATCATTTGTAGGGATCCC		TCTCAGAGCCTCGAGGATAT		590
TNF-RII	GAAATCCCAGGATGCAGTAG		TCAGGCCACTTTGACTGCAA		505

Effect of pretreatment by CY on intratumoral induction of TNF. We described in the previous report (24) that CY augmented endogenous TNF production in tumor bearing mice. We examined TNF induction following administration of the combination of CY with LPS or ONO-4007 around a tumor lesion. Seven days after inoculation of Meth A tumor, CY (100mg/kg) was administered i.p., and LPSp (400μg/kg) was administered i.d. 7 days later. The tumor was resected 90 minutes after LPSp administration, and TNF activity of the supernatant of the centrifuged homogenate samples was measured (Figure 1). Four fold augmentation of induction of endogenous TNF was obtained by CY plus LPSp compared to LPSp alone. We then tested whether several other chemotherapeutic agents could induce endogenous TNF because CY induced it slightly (Figure 1). CY (100mg/kg), melphalan (1.5mg/kg), or CPT-11 (44mg/kg) was administered i.p. 7 days after inoculation of Meth A tumor. The tumor was resected 7 days thereafter, and the TNF activity of the supernatant of the centrifuged homogenate samples was measured. As shown in Figure 2, the administration of CY or melphalan induced 26U/g and 9.5 U/g of TNF in the tumor, respectively, while the saline control did not (less than 2U/g). The change of TNF activity in tumor tissue and other organs (liver, spleen and serum) with time after injection of ONO-4007 (30mg/kg) was examined. As shown in Figure 3, when ONO-4007 was given, the endogenous TNF activity was observed in the serum, liver, spleen, and higher activity in the tumor tissues. Transient TNF activities were observed in the serum, liver and spleen one hour after ONO-4007 administration but were not observed after 3 hours. However, the strong induction of TNF was observed intratumorally in the group treated with ONO-4007 even 24 hours after the injection. The amount of endogenous TNF induced in tumor by the combination of ONO-4007 with CY was about 4 times higher than that without CY.

Table II. *Antitumor effect of i.d. administration of LPSp or i.v. administration of ONO-4007 combined with or without CY on three kinds of tumors, Meth A fibrosarcoma, MH134 hepatoma and Lewis lung carcinoma. Meth A cells ($2x10^5$), MH 134 cells ($2x10^5$) and Lewis lung carcinoma cells ($3x10^5$) were inoculated i.d. into the abdomen of BALB/c, C3H/He or C57BL/6 mice, respectively. Tumor bearing-mice were treated i.p. with CY (100 or 150mg/kg) on day 14 and administered i.d. with LPSp (400μg/kg) on day 21, 22, 23 and 24 or with ONO-4007 (30mg/kg) on day 21, 24 and 27. *Number of completely regressed (CR) mice/number of examined mice on day 30. **not done.*

	Meth A		MH134		Lewis lung	
	LPSp	ONO-4007	LPSp	ONO-4007	LPSp	ONO-4007
Control	0/12*	0/5	0/12	0/5	0/12	ND**
LPS	0/12	0/5	0/12	0/5	0/12	ND
CY	0/12	0/5	0/12	0/5	0/12	ND
CY+LPS	9/12	5/5	6/12	5/5	8/12	ND

Expression of mRNAs corresponding to Th1/Th2 cytokines in Meth A tumor tissue after administration of CY and TNF inducer. To analyze the cytokine expression pattern in tumor tissue treated with CY (150mg/kg) and ONO-4007 (30mg/kg), Th1 type cytokines (IL-1ß, TNF-α, IL-12 p40) and Th2 type

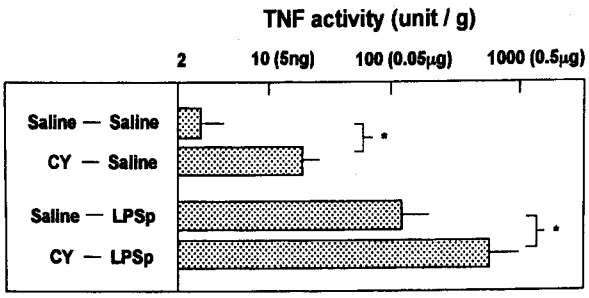

Figure 1. *Endogenous TNF activity in tumor after i.d. administration of LPSp in Meth A bearing BALB/c mice. Meth A bearing-BALB/c mice were treated i.p. with CY (100mg/kg) on day 7 and administered i.d. with LPSp (400μg/kg) on day 14. Tumors were homogenized and centrifuged as described in Materials and Methods. Columns and bars indicate mean values and standard deviations of the three individual samples. Significant differences (*:P<0.05, Student's t-test) from the saline control.*

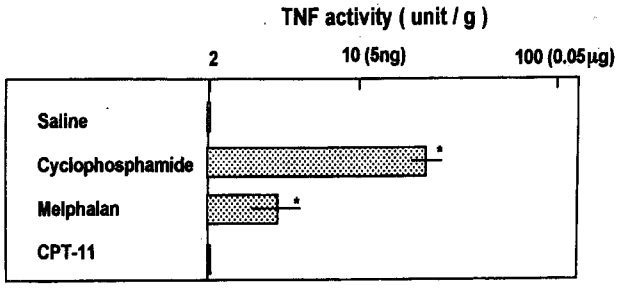

Figure 2. *Endogenous TNF activity in tumor by the chemotherapeutic agents. Meth A bearing BALB/c mice were treated i.p. with CY (100mg/kg), Melphalan (1.7mg/kg) or CPT-11 (44mg/kg) on day 7 and tumor tissues were resected on day 14. Tumors were homogenized and centrifuged as described in Materials and Methods. Columns and bars indicate the mean values and standard deviations of the three individual samples. Significant differences (*:P<0.05, Student's t-test) from the saline control.*

cytokines (IL-6, IL-10, TGF-ß) were examined as described in Materials and Methods. We used radar charts for presentation of the expression pattern of mRNA in tumor tissue (Figures 4, 5, 6). The radar charts in Figure 4 show the Th1 type cytokines (IL-1ß, TNF-α and IL-12 p40) on the right side and Th2 type cytokine (TGF-ß, IL-10, IL-6) on the left. Different expression patterns of Th1/Th2 type cytokines in Meth A were observed by CY combined with ONO-4007. The expression of mRNA of TGF-ß was decreased by administration of CY. IL-1ß, and TNF-α, and those of IL-12 p40 and IL-6 were increased by administration of ONO-4007, while that of TGF-ß was decreased by ONO-4007. Significant changes of IL-1ß and IL-6 were observed 1-3 hours after administration of CY followed by ONO-4007. The expression pattern of mRNA of Th1/Th2 cytokine in spleen is indicated in Figure 5. IL-10 and IL-6 were increased by the combination with CY followed by ONO-4007.

Expression of mRNAs corresponding to the molecule related to apoptosis in Meth A tumor tissue after treatment with CY and ONO-4007. Several chemotherapeutic agents and TNF are known to induce apoptosis of tumor cells. Thus, we examined the expression pattern of mRNA of apoptosis related molecules (Fas, Fas ligand, TNF receptor I and II) in tumor tissue after combination therapy. As shown in Figure 6, administration of ONO-4007 in combination with CY relatively decreased Fas ligand at 24 hours after injection, while there was no change of mRNA expression by CY alone. The results of change in the level of mRNA of either Th1 or Th2 cytokines and apoptosis related molecules in tumor tissue are summarized in Figure 7.

Discussion

We demonstrated that endogenous TNF at the dose of 0.25-0.75 μg/g following administration of CY could lead to

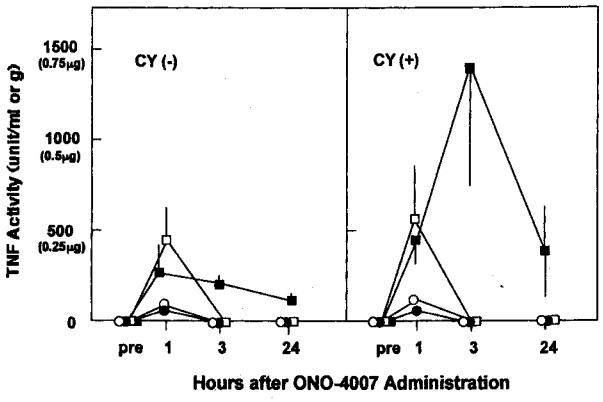

Figure 3. *Endogenous TNF activity in tumor, spleen, liver and serum after i.v. administration of ONO-4007 in Meth A bearing BALB/c mice. Meth A bearing BALB/c mice were treated i.p. with CY (150mg/kg) on day 7 and administered i.v. with ONO-4007 (30mg/kg) on day 14. Spleen, liver, tumor and blood were collected 0, 1, 3 and 24hours after ONO-4007 administration. Tissue samples were homogenized and centrifuged as described by Materials and Methods.* (○), *serum;* (●), *liver;* (□), *spleen;* (■), *tumor. Symbols and bars indicate mean values and standard deviations of three individual samples.*

complete tumor regression in Meth A, MH134 and Lewis lung carcinoma models (Figure 1 ,2 and Table II). Eggermont *et al* demonstrated that when the response rate exceeded 80% by isolation perfusion using TNF, the concentration of TNF in perfusate reached 2-5 μg/ml (27), and that of TNF in tumor over 0.1 μg/g (28). Taken together, a TNF concentrations of over 0.1 μg/g around a tumor lesion might be a prerequisite leading to complete tumor regression even in low immunogenic tumors.

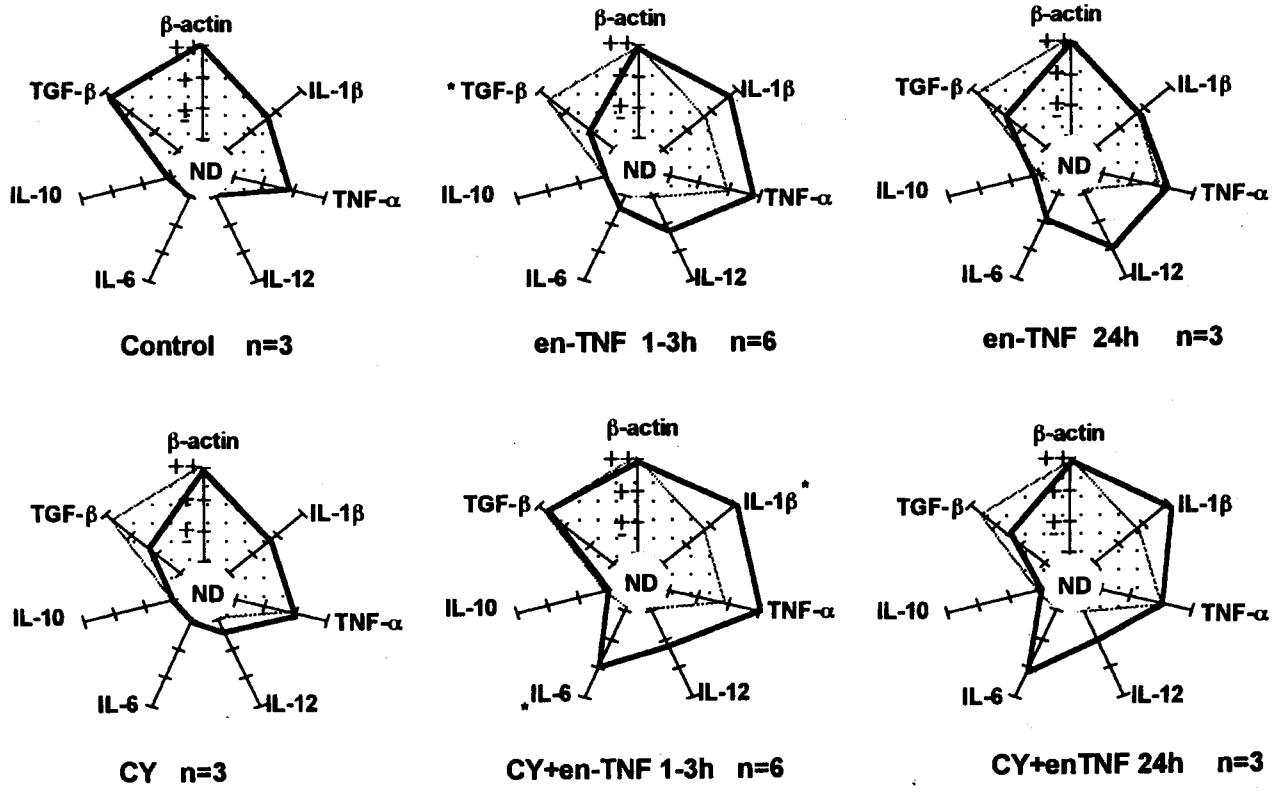

Figure 4. *Increase and decrease of expression of Th1/Th2 type cytokine messenger RNA in Meth A treated with i.v. administration of ONO-4007 and CY determined by RT-PCR Meth A fibrosarcoma bearing mice were administered CY (150 mg/kg) on day 7 and treated with ONO-4007 (30mg/kg) on day 14 after the tumor inoculation. Tumor tissues were taken, and expressions of messenger RNA of cytokines were detected by RT-PCR described in Materials and Methods. Relative amount of PCR product was compared with non-treated Meth A. Significant differences (*:P<0.05, Mann-Whitney's U test) from the control. Dotted area indicates control* pattern.

Endogenous TNF around a tumor lesion was observed 1 hour after the administration of LPSp or ONO-4007 and lasted for over 24 hours (Figure 3). In contrast, endogenous TNF was only transiently induced in serum, liver and spleen by the same administration. These results suggest that a high amount of TNF around a lesion is more important to obtain its strong antitumor response than systemic induction of TNF.

The reason LPSp or ONO-4007 could induce a higher amount of endogenous TNF around a tumor lesion than the systemical induction of TNF is not yet clear. Tumor vasculature seemed to play a crucial role in this, because TNF was demonstrated to augment significantly the expression of adhesion molecules such as vascular cell adhesion molecule-1 from endothelial cells of the tumor vasculature (29). Thus, expression or up-regulation of adhesion molecules is expected to increase the homing of granulocytes and lymphocytes (30) more specifically around a tumor lesions. Furthermore, Renard *et al* reported an early (within 3 hours) intraluminal and extraluminal polymorphonuclear leukocyte (PMN) accumulation within tumors, but not in normal skin, and later infiltration of macrophages and lymphocytes in tumors (31). Macrophages and/or PMN which infiltrated and then are activated by cytokines can be the main effector cells assuring continuous production of endogenous TNF around a tumor lesion. Therefore, the mechanism of augmented and prolonged production of TNF around a lesion may partly ascribed to continuous activation of macrophages and/or PMN by TNF which are accumulated around lesions after the administration of LPSp or ONO-4007.

As shown in Table II, CY is an essential property to obtain complete tumor regression by LPS and/or ONO-4007. Mokyr *et al* suggested that the mode of action of melphalan leading to complete regression of MOPC-315 tumor is suppression of the production of TGF-ß (15) and induction of cytotoxic T lymphocytes by inducing Th1 type cytokines such as IL-12, interferon-γ (17) and TNF-α(16). Since both CY and melphalan belong to the same category (alkylating agents), it is likely that the mechanism operating melphalan is also involved in the antitumor effect of CY. In fact, the production of TGF-ß around the tumor lesion was suppressed 7 days after the administration of CY (Figure 4). Moreover, since

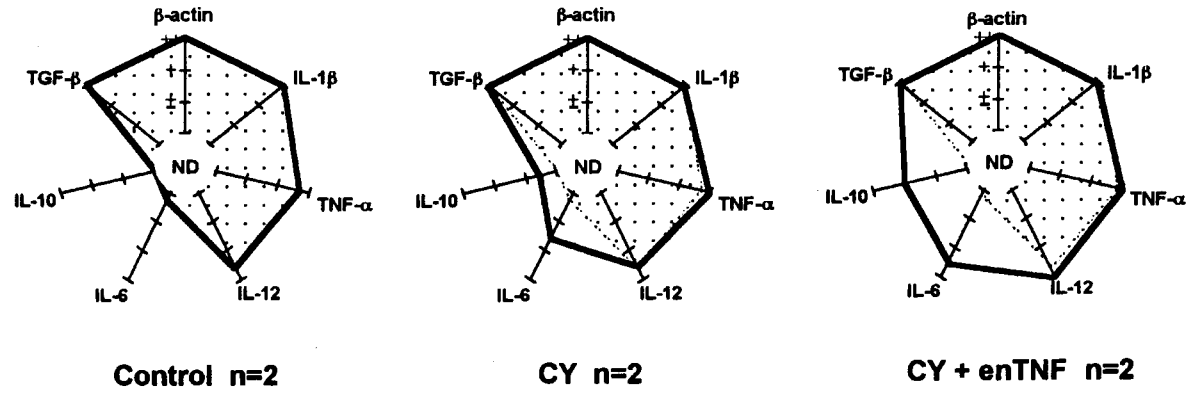

Figure 5. *Increase and decrease of expression of Th1/Th2 type messenger RNA in spleen treated with i.v. administration of ONO-4007 and CY determined by RT-PCR Meth A fibrosarcoma bearing mice were administered CY (150 mg/kg) on day 7 and treated with ONO-4007 (30mg/kg) on day 14 after the tumor inoculation. Tumor tissues were taken, and expressions of messenger RNA of cytokines were detected by RT-PCR described in Materials and Methods. Relative amount of PCR product was compared with non-treated Meth A. Dotted area indicates control pattern.*

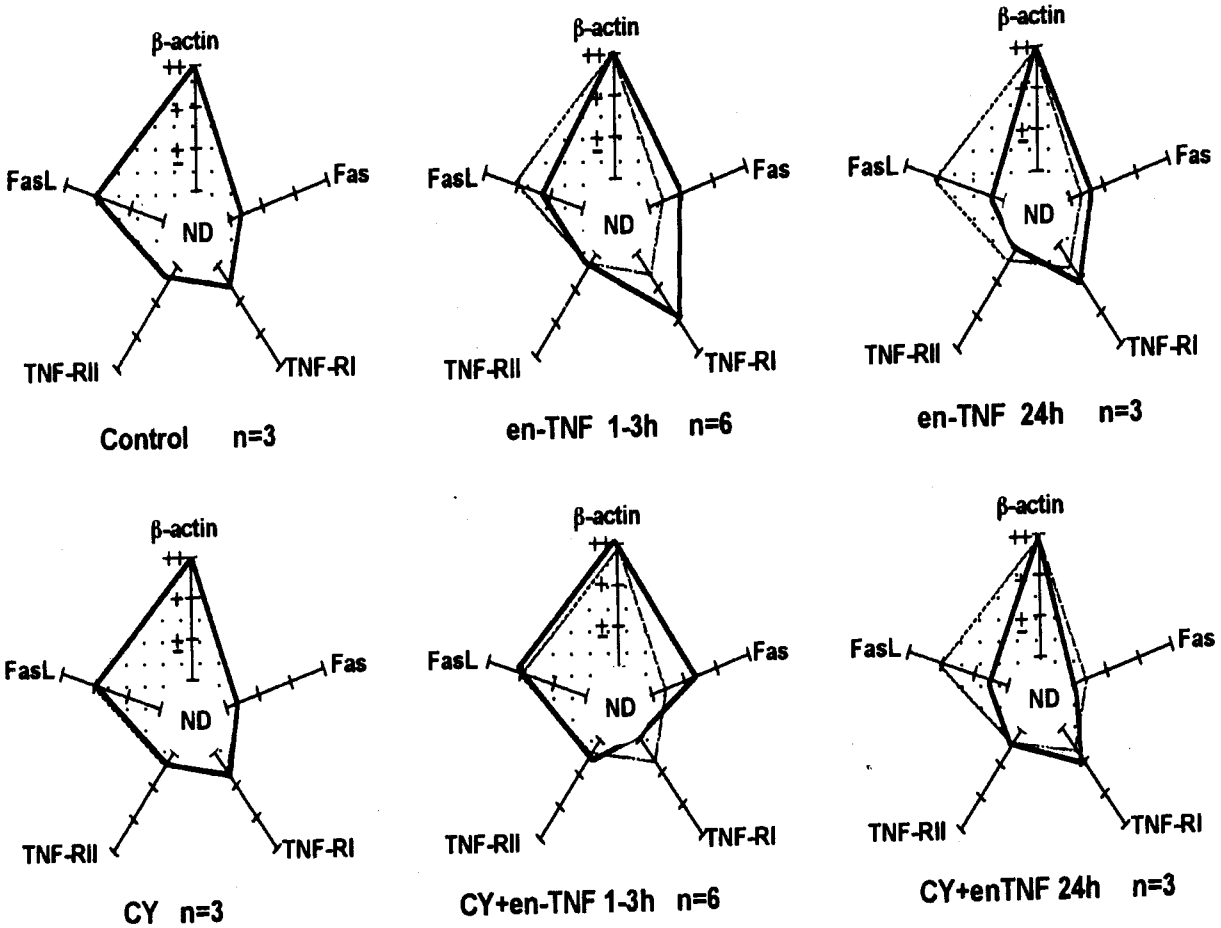

Figure 6. *Increase and decrease of expression of apoptosis related protein messenger RNA in Meth A treated with i.v. administration of ONO-4007 and CY determined by RT-PCR Meth A fibrosarcoma bearing mice were administered CY (150 mg/kg) on day 7 and treated with ONO-4007 (30mg/kg) on day 14 after the tumor inoculation. Tumor tissues were taken, and expressions of messenger RNA of cytokines were detected by RT-PCR as described in Materials and Methods. Relative amount of PCR product was compared with non-treated Meth A. Dotted area indicates control pattern.*

		Injection of ONO-4007		
		Pre	1-3h	24h
None	Increase		IL-1β TNF-α IL-12	IL-12 IL-6
	Decrease		TGF-β*	Fas L
CY	Increase		IL-1β* TNF-α IL-6* IL-12	IL-1β IL-12 IL-6
	Decrease	TGF-β		TGF-β Fas L

Figure 7. *The summary of the changed mRNA of Th1/Th2 cytokines and apoptosis related molecules in Meth A tumor. Significant differences (*: P<0.05, Mann-Whitney's U test) from the control.*

TNF was actually induced around the lesion by CY alone, as shown in Figures 1 and 2, the host's immune status with this agent might change from Th2 dominant status induced by the tumor, to Th1 dominant status, followed by modification of responsiveness of LPS and leading to tumor regression. However, as shown in Figure 4, CY alone could not change the pattern of cytokine expression from Th2 to Th1; this shift occurred only after administration of ONO-4007. ONO-4007 alone also induced the change of cytokine expression, however, in our experiment, only IL-6 was shifted by the combined treatment of CY and ONO-4007. The significance of IL-6 leading to tumor regression is not yet known, however, since IL-6 is reported to show an antitumor effect in some tumors (32) it is possible that IL-6, as a Th2 type cytokine, together with TNF-α or IL-1ß could form a cytokine network which is effective in tumor regression. More precise analyses on the kind of cytokines are required to obtain the strongest antitumor effects.

As shown in Figure 6, the expression of Fas-ligand (Fas-L) was suppressed by the combined treatment of CY and ONO-4007. This expression demonstrated one mechanism to allow tumors to escape immune surveillance (33); suppression of the expression of Fas-L by these combinations might thus be another way of achieving strong antitumor effects.

From all the results presented so far, the crucial points for establishment of a novel antitumor therapy based on the administration of TNF can be summarized as follows: a) TNF must be present at doses of over 0.1 μg/g around a tumor lesion. b) a chemotherapeutic agent such as CY or melphalan might be essential to strengthen the antitumor effects of TNF, and one way to select the best agent is by whether it can alter the pattern of cytokine expression from Th2 to Th1, especially around the tumor lesion. Recently, the biological significance of innate immunity for host defense has been reevaluated (34). In this sense, effective antitumor therapy may only be established by integration of innate immunity with the structural specificity of acquired immunity. Thus the role of chemotherapeutic agents may be reassessed as a coupler of innate immunity with acquired immunity, as demonstrated here. If these points are true, we will be able to establish a novel antitumor therapy using cytokines such as TNF with chemotherapeutic agents; this will be based on the previous observation of the immunological status around the tumor lesion of a patient, and focused especially on the amount of induced endogenous TNF and the shift in the pattern of cytokine expression. This means that in the near future, therapy based on TNF may be possible to open a new era of original therapeutic protocol depending on an individual tumor as well as the host's conditions.

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