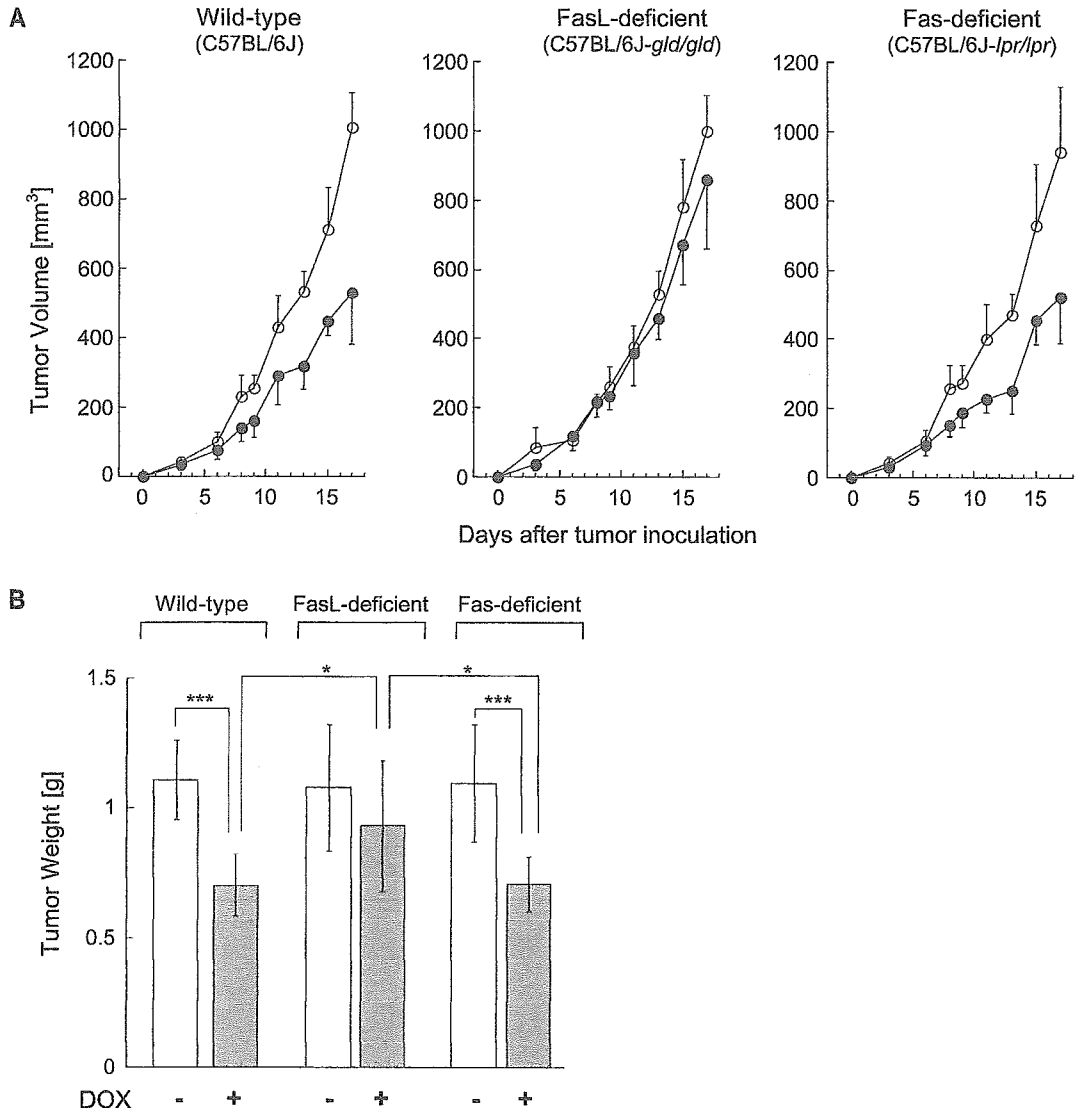


Fig. 3. Antitumor chemotherapeutic effect of DOX against 3LL tumor in C57BL/6 mice. (A) Effect of DOX treatment in 3LL tumor growth. 3LL cells ($5\times10^5$) were inoculated s.c. on day 0. Saline (for control group [○]), DOX (2.5 mg/kg [●]), was administrated i.p. on days 1–5, 7–11, and 14–16. The tumor volume was measured on the days indicated. Each group consisted of eight mice; bars, S.D. (B) Tumor weights at day 17. Mice were sacrificed, and the tumor weights were measured. All mice survived at the end of the experiment. $^*P<0.05$, $^{***}P<0.005$ in comparison between the indicated groups; bars, S.D. No significant difference was observed in wild-type control vs. *gld* control, wild-type control vs. *lpr* control, *gld* control vs. *gld* DOX, and wild-type DOX vs. *lpr* DOX.

Next, we examined the antitumor effect of DOX on an established tumor in the syngeneic models. DOX was administered i.p. at days 8 and 14. As shown in Fig. 4A and 4B, DOX showed no significant antitumor effect in *gld* mice, while it inhibited the tumor growth in wild-type mice. Furthermore, Fas expression in vivo was increased in two out of three 3LL solid tumors by only once injection of DOX (Fig. 5). As *lpr* mice were used as hosts for this experiment, the mRNA of Fas was not derived from the host cells.

These results suggest that Fas and FasL play an essential role in the antitumor effect of DOX against 3LL solid tumor possibly through enhanced Fas expression in the tumor cells and host immune response.

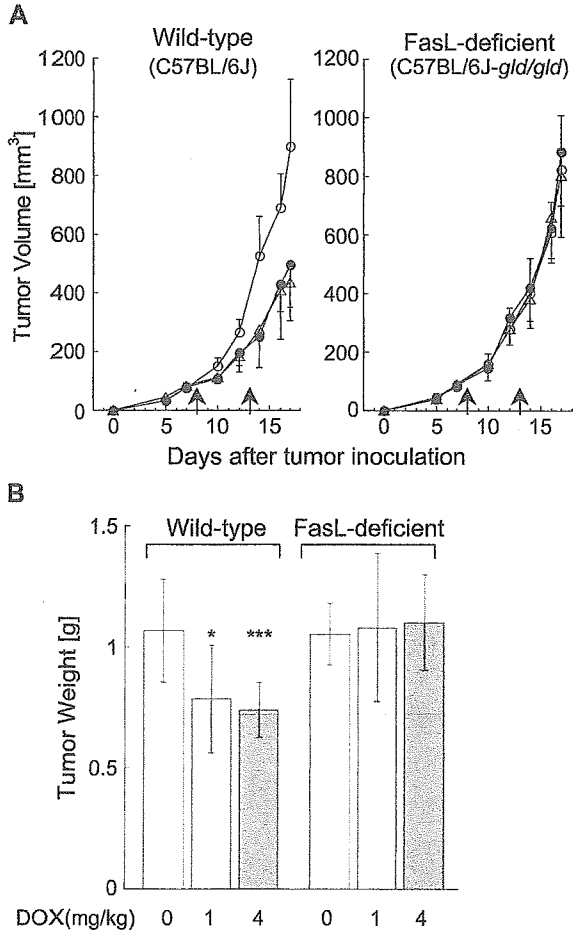


Fig. 4. Antitumor chemotherapeutic effect of DOX against established 3LL tumor in C57BL/6 mice. (A) Effect of DOX treatment in 3LL tumor growth. 3LL cells ($5\times10^5$) were inoculated s.c. on day 0. Saline (for control group [○]), DOX (1 mg/kg [•]), DOX (4 mg/kg [△]) was administrated i.p. on days 8 and 14. The tumor volume was measured on the days indicated. Control group and DOX (1 mg/kg) group consisted of seven mice, and the DOX (4 mg/kg) group consisted of six mice; bars, S.D. (B) Tumor weights on day 17. Mice were sacrificed, and the tumor weights were measured. All mice survived at the end of the experiment. *$P<0.05$, ***$P<0.005$ in comparison with control group; bars, S.D. No significant difference was observed in wild-type control vs. *gld* control, *gld* control vs. *gld* DOX (1 mg/kg), and *gld* control vs. *gld* DOX (4 mg/kg). Results show one representative experiment of the three performed.

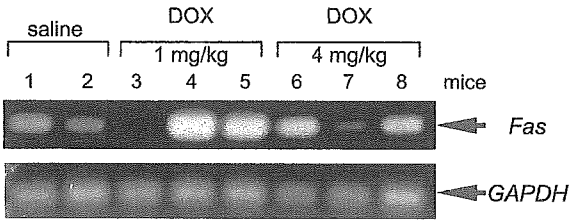


Fig. 5. Expression of Fas mRNA in 3LL solid tumor. C57BL/6-*lpr* mice bearing 3LL tumor were treated with saline (mice 1 and 2), 1 mg/kg of DOX (mice 3, 4, and 5), 4 mg/kg of DOX (mice 6, 7, and 8); after 24 h, the mice were sacrificed, and the solid tumors were obtained. mRNA expressions of Fas and GAPDH were analyzed as detailed in Materials and methods.

## 4. Discussion

In the present study, we have found that DOX does not show antitumor effect against 3LL solid tumor in FasL-deficient C57BL/6-*gld* mice. In addition, DOX-induced expression of Fas was detected in vivo as well as in vitro. Although there was significant reduction in Fas expression in some DOX-treated mice, it is possible that the Fas-expressing 3LL cells were efficiently eliminated in vivo. These evidences suggest that DOX inhibits the tumor growth through the host immune response in this syngeneic model. It has been considered that there are several mechanisms as in vivo antitumor effect of DOX: (i) direct antiproliferative effect, (ii) apoptosis of tumor cells by autocrine signaling via Fas and FasL [21–23], and (iii) selective elimination of immune suppressor cell activity and subsequent augmentation of immune response [15,16]. DOX showed no significant inhibition of the growth of 3LL solid tumor in *gld* mice, indicating that the neither mechanism (i) nor (ii) is involved in the antitumor effect of DOX in this syngeneic model. Mechanism (iii) is also neglected because inhibition of the tumor growth was observed in *lpr* mice but not in *gld* mice. Therefore, in this model, it is considered that DOX exerted in vivo antitumor effect against 3LL solid tumor through enhanced Fas expression in the tumor cells and host immune response. Kalechman et al. [24] reported that AS101, synthetic immunomodulator that enhances Fas expression, shows antiproliferative effect against

B16 melanoma in vivo through a host immune response. Moreover, Micheau et al. [9] reported that cisplatin increases Fas expression and sensitivity to Fas-dependent cytolysis by peripheral blood leukocytes in human colon HT29 cells. Therefore, these reports support our findings that the antitumor effect of DOX, at least in part, depends both on Fas expression in tumor cells and on host immune response. Especially in the 3LL-syngeneic model, inhibition of the tumor growth mainly depends on CTL-mediated cytolysis via Fas because significant therapeutic effect of DOX was not observed in FasL-deficient *gld* mice. Moreover, DOX-pretreated 3LL cells were significantly killed in vitro by the splenic T cells prepared from 3LL-bearing C57BL/6 mice. Antitumor drug-induced Fas expression and enhanced sensitivity to Fas-mediated apoptosis were also observed in several human cancer cell lines as well as in 3LL cells [9–11], which suggested that the antitumor drug-induced Fas expression and subsequent host immune response could also take place in human malignancies.

We report here for the first time that an antitumor drug used clinically induces Fas expression in solid tumor in vivo, and that the Fas expression contributes to the chemotherapeutic effect. It was reported that administrations of certain antitumor drugs augmented host immune responses against tumors [15,16]. The Fas expression might disrupt immune evasion of tumor cells and be one of possible mechanisms for the augmentations of host immune responses. Thus, it should be considered that hematologic toxicity at higher doses of drug could reduce the host immune response and thereby reduce the antitumor effect. In addition, this theory could be one of possible explanations for efficacy of cancer chemotherapy using low-dose antitumor drugs [25,26]. Furthermore, the combination of antitumor drugs with biological response modifiers that activate tumor immunity or with adoptive immunotherapy using tumor-specific CTLs would improve cancer treatment.

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