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that was maintained during their presence, though its magnitude gradually declined with time. The sustained  $[Ca^{2+}]_i$  rise induced by these drugs was reversibly blocked by exposure to a  $Ca^{2+}$ -free solution containing EGTA (500  $\mu$ M). When applied 30 s after removal of external  $Ca^{2+}$ , muscarine, but neither nicotine nor KCl, caused an early rise in  $[Ca^{2+}]_i$  transiently.

7. Muscarine-induced  $[Ca^{2+}]_i$  rise in  $Ca^{2+}$ -free solution was partially reduced by proceeding application of caffeine or by treatment with ryanodine plus caffeine.

8. Intracellular application of inositol 1, 4, 5-trisphosphate caused a transient increase in  $[Ca^{2+}]_i$  and inhibited the following  $[Ca^{2+}]_i$  response to muscarine without affecting the responses to nicotine and a depolarizing pulse.

9. Muscarine evoked membrane depolarization following a transient depolarization and brief hyperpolarization in most cells tested. There was a significant positive correlation between the amplitude of the depolarization and the magnitude of the sustained rise in  $[Ca^{2+}]_i$ . Muscarine-

induced sustained  $[Ca^{2+}]_i$  rise was much greater in the current-clamp mode than that in the voltage-clamp mode.

10. The sustained  $[Ca^{2+}]_i$  rise and  $Mn^{2+}$  influx in response to muscarine were suppressed by a voltage-dependent  $Ca^{2+}$  channel blocker, methoxyverapamil.

These results are summarized as follows : 1) catecholamine secretion and  $[Ca^{2+}]_i$  rise induced by muscarinic agonists may be mediated through  $M_1$ , or  $M_1$  and  $M_3$  muscarinic receptor subtypes in adrenal chromaffin cells of the guinea pig. 2) muscarinic receptor activation causes not only extracellular  $Ca^{2+}$  entry, but also  $Ca^{2+}$  mobilization from inositol 1, 4, 5-trisphosphate-sensitive intracellular stores, which are suggested to overlap those sensitive to caffeine. 3) either voltage-dependent  $Ca^{2+}$  channels or non-selective cation channels may function as the  $Ca^{2+}$  entry pathways activated by muscarinic receptor in guinea pig adrenal chromaffin cells. 4) ATP causes increase in catecholamine secretion exclusively by increasing the entry of extracellular  $Ca^{2+}$ .

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Study of antitumor immunity induced by  
neocarzinostatin derivative, zinostatin stimalamer

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Zinostatin stimalamer (ZSS) is a new anti-cancer agent derived from neocarzinostatin (NCS) which is synthesized by conjugation of one molecule of NCS and two molecules of poly(styrene-co-maleic acid). ZSS exhibited potent

*in vitro* and *in vivo* antitumor activity in preclinical experiments, and clinical trials of the intra-arterial administration of ZSS with iodized oil on hepatocellular carcinoma showed potent anti-tumor activity.

The effect of ZSS and NCS on antitumor resistance was investigated and it was found that pretreatment with both drugs suppressed the growth of Meth A tumors in Balb/c mice and induced tumor eradication when given separately by single administration at therapeutic doses between 1 day and 4 weeks before tumor transplantation. The findings that the cytotoxic activity of these drugs was not detected *in vivo* at the time of tumor transplantation and that tumor regression was preceded by a period of transient growth suggested that tumor regression was due to host-mediated antitumor activity induced by these drugs. Pretreatment with ZSS or NCS also suppressed the growth of Colon 26 carcinoma and Sarcoma 180. The finding that NCS showed the same effect as ZSS suggests that poly(styrene-co-maleic acid) is not essential for the induction of host-mediated antitumor activity. Furthermore, apo-ZSS, which lacks cytotoxic activity, did not induce antitumor activity. Collectively, it is suggested that the cytotoxic effect of ZSS involves the induction of host-mediated antitumor resistance.

In athymic Balb/c nu/nu mice, pretreatment with ZSS or NCS did not induce tumor eradication, suggesting that mature T lymphocytes play an important role in tumor eradication. Challenging Meth A was rejected without transient growth in mice which had been cured of Meth A, but challenging Colon 26 was not, showing that anti-Meth A resistance was augmented selectively in the Meth A-eradicated mice.

Splenocytes from Meth A-bearing mice pretreated with these drugs showed tumor neutralizing activity beginning 14 days after tumor transplantation. Tumor neutralizing activity was only induced after Meth A transplantation. The effector cells of tumor neutralizing activity was

Thy1.2<sup>+</sup> nylon wool column-passed T lymphocytes. However, no significant augmentation of cell-mediated cytotoxic activity of splenocytes from Meth A-eradicated mice was observed *in vitro*. ZSS or NCS given on day -3 transiently decreased the number of spleen cells, with an increased percentage of T cells but decreased those of B cells and macrophages. These changes were observed only before but not in the period of tumor regression. B cells increased in inguinal lymph nodes of Meth A-bearing control mice, but such elevation of B cells was attenuated by ZSS or NCS pretreatment. Although histological examination of tumor nodules showed the presence of only few host immune cells in the tumor tissue of ZSS or NCS-pretreated mice, the area of tumor cell death was already extensive on day 7 and expanded thereafter. From these results, it was suggested that these drugs might affect the precursor cells responsible for tumor eradication and that tumor cell death was possibly due to cytokine(s) produced by the antitumor effector cells.

*In vivo* depletion experiments using antibodies or carrageenan showed that antitumor effector cells for tumor eradication are Thy1.2<sup>+</sup>/Lyt2.2<sup>+</sup> and that at least a part of them are asialo GM1<sup>+</sup>. Thy1.2<sup>+</sup>/Lyt2.2<sup>+</sup>/asialo GM1<sup>-</sup> cells are important in generation of the antitumor effector cells. L3T4<sup>+</sup> T cells are also involved in initiation of tumor regression and in tumor eradication.

Antitumor effect of posttreatment with ZSS was suppressed partially by *in vivo* depletion of T cells. In conclusion, it was suggested that ZSS might exhibit antitumor activity by augmenting host-mediated antitumor resistance as well as its intrinsic cytotoxic activity.

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