ANTITUMOR MECHANISMS OF INTRADERMAL ADMINISTRATION OF LIPOPOLYSACCHARIDE WHICH INDUCED HIGH ANTITUMOR EFFECT ON EXPERIMENTAL TUMORS DUE TO ITS SLOW RELEASE OF EFFECTOR MOLECULES.

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Introduction

Lipopolysaccharide (LPS), an endotoxin of Gram negative bacteria, which shows strong antitumor effect on experimental tumors, has been administered to cancer patients as the major component of Coley's toxin (1). Despite its high antitumor effect, however, it has been clinically applied only in a limited number of cases, because of its severe side effects such as endotoxic shock especially when administered intravenously (iv) (2).

We previously reported that intradermal (id) administration of LPS induced activation of reticuloendothelial cells as indicated by carbon clearance, and showed a marked antitumor effect against allogeneic murine tumors at the same level as iv administration of LPS (3). Since then no report except ours has been published concerning id administration of LPS to experiment animals. On the other hand, we reported that the antitumor effect of biological response modifiers (BRMs), including LPS, can be evaluated by the degree of inflammation in which the tumor necrosis factor (TNF) produced can be taken as its index (4). The antitumor effect of id LPS is believed to be ascribable to a mechanism associated with its slow and continuous release which induces TNF production. In this paper, the action mechanism and antitumor effect of id administration of LPS is reported.

Results

Low molecular weight LPS well standardized were used to these studies (5). To determine the dynamic response in the skin after LPS-administration, the change with time in the amount of LPS remaining in skin and also that of free TNF activity, an indicator of the macrophage activation, were examined. TNF activity was measured by cytotoxicity assay against L929 cells in the presence of actinomycin D (1μg/ml). Histological change with time in the skin was also observed. LPS amount was measured with Toxicolor (a kit preparation of Limulus test; Seikagaku Co. Tokyo, Japan). LPS was found to decrease in the skin with a half-life of 90 minutes during the first 5 hours and thereafter with a half-life of 12 h (Fig.1). Even 48 hours after LPS injection, a significant amount of LPS (more than 1% of the dose) could be detected in skin, presumably enough to induce free as well as precursor TNF. Serum LPS was not detected 15 minutes after LPS injection, then significant amount of LPS (less than 0.1% of the dose) was found to decrease in serum as well as dermal LPS (Fig.1).

It is conceivable that the decrease of LPS during the early stage is ascribable to a leak into blood vessels, leading to the activation of inflammatory cells there in the same way as with small amount of intravenously administered LPS. The amount of LPS remaining in the skin at a later stage may have expressed various functions. As shown in Fig.2, free TNF was produced in the local site of skin by LPS administration. A detectable amount of TNF was produced in the skin 3 h after LPS treatment and the maximum activity was observed after 24 h. TNF activity decreased gradually, but was significantly detectable at the skin even at 48 hours. Iv LPS injection can induce TNF production at its maximal amount at 1 h and not later than 3 h in serum after the administration. Samples of removed skin with time after LPS administration were analyzed histologically. A typical event observed was the inflammatory cell accumulation. Neutrophiles were assembled around the blood vessels of the skin 6 hrs after LPS injection. After 24-48 hrs, numerous inflammatory cells, mainly macrophages and neutrophiles were seen in the dermis and tela subcutenea. Relative amount of the assembled cells is indicated at the bottom line in Fig.2.

The antitumor effect of intradermally administered LPS was examined using a syngeneic tumor, Meth A fibrosarcoma. Meth A tumor is an experimental model tumor with which antitumor effects have been shown with various BRMs including LPS. Daily LPS were given intradermally starting from day 5 after tumor inoculation for 4 consecutive days. A high therapeutic effect, including 75% of complete regression was observed in a group treated with 400μg/kg of LPS. We tested the antitumor activity of id administration of LPS alone against MH134 hepatoma and Lewis lung carcinoma starting soon after tumor inoculation. LPS was injected id daily for successive days from day 5 after the inoculation. Tumor growth was significantly suppressed by the id treatment with LPS in both tumors, although no complete regression was observed even in the group treated with 400μg/kg of LPS. To enhance the antitumor effect of administration of LPS, cyclophosphamide (CY) was given once prior to the id administraton of LPS, remarkable antitumor effects were obtained, with complete regression being observed in Meth A, MH134 and Lewis

lung carcinoma (Fig.3). Pretreatment of anti-TNF antibody reduced this effect of id LPS.

Discussions

The effect of i.d. administration of LPS is shown in two ways: one is the continuous assembling of reticuloendothelial cells which are activated by LPS in the region of administration to induce the continuous production and release of either free TNF or TNF-primed cells (effector molecules) into the bloodstream. The other is the slow release of LPS from its injected site into the bloodstream and the continuous production of TNF-primed reticuloendothelial cells in the blood. Free TNF produced in skin lesion (Fig.2) probably released into the bloodstream over a long period and could prime reticuloendothelial cells in the bloodstream. TNF contribution from skin lesion could also participate in this event, though the amount is small. Contrary to the iv route, the major amount of LPS administered intradermally stays long there continuously for more than 48 hrs.

Free TNF and cell membrane-bound TNF (TNF precursor) are main effector molecule for the antitumor effect by LPS. This membrane-bound TNF has a similar cytocidal activity against tumor cells to that of free TNF (6). In fact, in this experiment, pretreatment of anti-TNF antibody was seen to suppress the characteristic hemorrhagic necrosis and also the therapeutic effects of id LPS. In MH134 and Lewis lung tumors, both of which are known to be potently metastatic, mice still survived on day 120 after the tumor inoculation when treated with id administration of LPS, and no metastatic lesions in lung or lymph node were observed. We considered these mice to be completely cured. In contrast, metastases were observed in all control mice and in the groups treated either with LPS or CY alone. Even mice which received the combined treatment of LPS-CY at a later period after the tumor inoculation when a metastasis (tumor diameter more than 15 mm) was established in lung or lymph node showed complete regression. These results indicate the usefulness of id administration of LPS in clinical application simply due to its slow release, when administered alone or in combination with a favorable chemotherapeutic such as CY.

Fig.1 Fate of LPS in the Skin and Serum with Time after Intradermal Administration

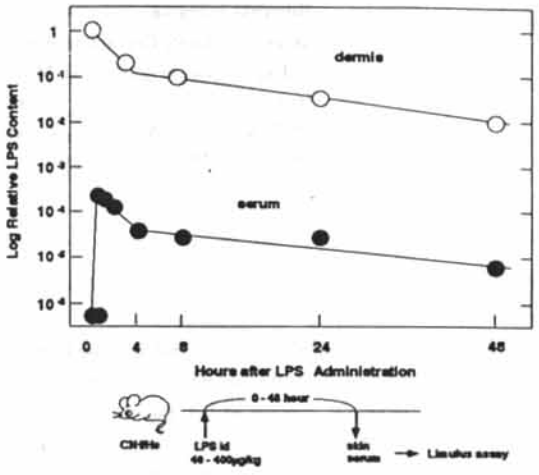


Fig.2 TNF Activity in the Skin with Time after Intradermal Administration of LPS

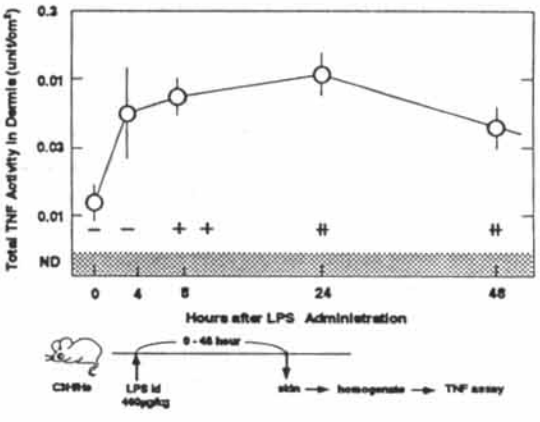
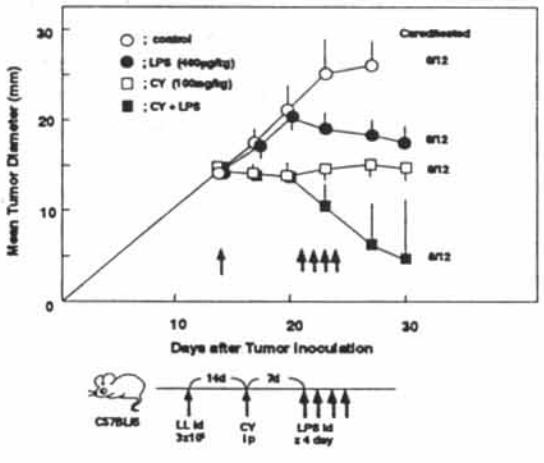


Fig.3 Antitumor Effect of LPS Combined with CY on Lewis Lung Carcinoma



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