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Mechanism of Lipopolysaccharide-Induced Tumor Necrosis: Requirement for Lipopolysaccharide-Sensitive Lymphoreticular Cells

DANIELA N. MÄNNEL, DAVID L. ROSENSTREICH, AND STEPHAN E. MERGENHAGEN*

Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20014

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Lipopolysaccharide (LPS) induces rapid necrosis of intradermal fibrosarcomas in mice. The mechanism(s) by which LPS produces tumor necrosis has been investigated using histocompatible LPS-sensitive (C3H/HeN) and LPS-resistant (C3H/HeJ) mouse strains. C3H/HeN- or C3H/HeJ-derived fibrosarcomas were necrotized by LPS when they were grafted onto C3H/HeN mice but were not affected when growing on C3H/HeJ mice, indicating that LPS does not act directly on the tumor itself. In contrast, lethally X-irradiated C3H/HeJ mice exhibit necrosis of their tumors when reconstituted with C3H/HeN bone marrow cells, whereas C3H/HeN mice no longer exert LPS-induced tumor necrosis after the adoptive transfer of C3H/HeJ bone marrow cells. These findings clearly indicate that LPS produces necrosis of tumors by activating host lymphoreticular cells.

Lipopolysaccharide (LPS, endotoxin)-induced tumor necrosis is a well-known phenomenon (8, 13, 16), but the mechanism(s) by which this effect is mediated is not well understood. It has been suggested that LPS acts directly on the tumor cells (5) or that necrosis is due to vasoconstriction and intravascular coaggulation within the tumor (1). More recently it has been reported that LPS induces the release of a tumor necrosis factor from macrophages in vivo (7, 10). This finding as well as others suggests that LPS exerts an influence on tumors by the production of humoral mediators (6).

C3H/HeJ mice are resistant to all known effects of LPS, whereas the closely related, histocompatible C3H/HeN mouse strain is LPS sensitive. In addition, it is possible to render C3H/HeJ mice sensitive to LPS by the adoptive transfer of C3H/HeN spleen or bone marrow cells and render C3H/HeN mice LPS resistant by the reciprocal transfer (9, 14). Using C3H/HeJ mice and the adoptive transfer model system, we have investigated the mechanism by which LPS induces tumor necrosis. Our data suggest that LPS does not act directly on the tumor cells or on the host vasculature. Instead, our findings are consistent with the hypothesis that LPS mediates tumor necrosis solely by an effect on lymphoreticular cells.

Female mice, 6 to 12 weeks of age, were obtained from the Division of Research Services, National Institutes of Health (C3H/HeN) and from the Jackson Laboratory, Bar Harbor, Maine (C3H/HeJ). *Escherichia coli* K-235 LPS was prepared by the phenol-water extraction method of McIntire et al. (11). Bone marrow cells were obtained by gently grinding and then rinsing femurs and tibiae of the donor mice in cold RPMI medium (GIBCO Laboratories, Grand Island, N.Y.). Bone fragments were removed by allowing them to settle for 5 min; the cells were washed and then suspended in medium to a concentration of 10^8 cells per ml. Cell viability exceeded 90% as determined by the exclusion of trypan blue dye. Mice received 850 R of X-irradiation and were reconstituted within 6 h by tail vein injection of 10^7 bone marrow cells. Four weeks after reconstitution, the mice were used in the tumor experiments. In a study to be reported separately, 850 R of X-irradiation completely abrogated the ability of these mice to form splenic colony-forming units (Michalek et al., manuscript in preparation).

Tumors (fibrosarcomas) were induced by injecting mice intradermally with 1 mg of 3-methylcholanthrene in 0.2 ml of trioctanoin (tricaprylin; Sigma Chemical Co., St. Louis, Mo.) on both sides of the back. For tumor passage, solid pieces were inoculated subcutaneously as previously described (2). Experimental tumors were induced by inoculating single tumor cell suspensions which were obtained from minced tumor fragments by enzymatic digestion (2). Tumor cells (10^6) were injected intradermally, and after

14 days the mice with tumors (7 ± 1 mm in diameter) were injected intraperitoneally with 50 μg of LPS. Tumor necrosis was determined by visual inspection on days 1, 3, 5, and 7. The results on day 3 are reported because the necrotic response did not change later on. Only tumors with >25% necrosis of the surface were scored as positive. In certain experiments, an established fibrosarcoma (tumor 1023) was used that had been induced in a C3H/HeIcr male mouse (12). All statistical analyses were performed using the Fischer test (4).

To determine if the derivation of the tumor from either endotoxin-sensitive or -resistant mice would influence its sensitivity to LPS-induced necrosis in vivo, tumors were induced with methylcholanthrene in C3H/HeN and C3H/HeJ mice and then tested in both C3H/HeN and C3H/HeJ hosts for their LPS sensitivity. A C3H/HeN (LPS-sensitive)-derived tumor was necrotized in >70% (11/15) of the animals after injection of 50 μg of LPS when carried on the endotoxin-sensitive C3H/HeN mouse strain; only 7% (1/14) of the tumors were necrotized on C3H/HeJ mice ($P < 0.001$; control tumor-bearing mice of both strains, injected with saline, showed no necrosis [0/5 for each strain]). In a similar type of experiment employing a transplanted C3H/HeJ (LPS-resistant)-derived tumor, again >60% (5/8) of the endotoxin-sensitive mice (C3H/HeN) showed tumor necrosis after injection of 50 μg of LPS, whereas none of the tumors (0/7) on endotoxin-resistant (C3H/HeJ) mice developed necrosis ($P < 0.02$; in saline-injected controls, 0/5 of C3H/HeN and 1/4 of C3H/HeJ mice showed necrosis). To determine if the inability of C3H/HeJ mice to necrose transplanted tumors was due to an abnormal tumor growth pattern in these mice, the growth curves of a tumor derived from a LPS-sensitive mouse were compared in C3H/HeN and C3H/HeJ mice. The growth curves of the 1023 (C3H/HeIcr) fibrosarcoma after intradermal injection of C3H/HeJ and C3H/HeN mice with 10^6 cells do not show any difference in growth rates of this tumor in either group of mice (Fig. 1). These experiments indicate that the origin of the tumor does not influence its susceptibility to endotoxin-induced necrosis and also suggest that LPS does not act directly on the tumor itself.

Since the ability to necrotize tumors with LPS is dependent upon the LPS sensitivity of the host and not on the origin of the tumor, we attempted to alter host sensitivity to LPS by X-irradiation and reconstitution of the irradiated recipient with bone marrow cells from either LPS responder or nonresponder mice. The results obtained with these chimeric mice are shown in Tables 1 and 2. The transfer of 10^7 bone marrow cells from LPS responder mice (C3H/HeN) to irradiated C3H/HeJ mice rendered the latter (>90%) susceptible to the tumor-necrotizing influence of LPS when engrafted with either the C3H/HeJ- or C3H/HeN-derived fibrosarcoma. Reconstitution with autologous bone marrow cells did not alter the responsive state of the host. On the other hand, C3H/HeN mice could be rendered almost totally nonresponsive to tumor necrosis with LPS by adoptive transfer of C3H/HeJ bone marrow cells, since only 11 to 20% of these chimeras exhibited LPS-induced tumor necrosis when engrafted with either type of tumor. Thus, adoptively transferred bone marrow cells caused sig-

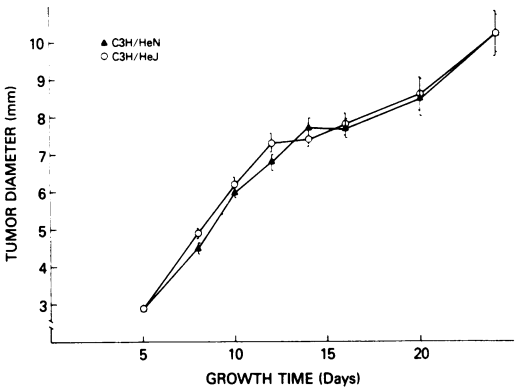

FIG. 1. *Growth rate curves of fibrosarcoma 1023 in C3H/HeN and C3H/HeJ mice. A total of 10^6 tumor cells in 0.05 ml of RPMI 1640 were injected intradermally on day 0 in the backs of the animals. Each point represents the mean tumor diameter ± standard error determined from a group of 30 animals.*

TABLE 1. *Susceptibility of a C3H/HeN-derived fibrosarcoma to LPS-induced necrosis when implanted in C3H/HeN, C3H/HeJ, and chimeric mice*

Recipient[a]	Source of bone marrow for reconstitution	Mice with necrotic tumors[b]/ total mice injected[c]	*P*
C3H/HeJ		2/14	
$C3H/HeJ_X$	C3H/HeJ	2/12	<0.001
$C3H/HeJ_X$	C3H/HeN	15/16	
C3H/HeN		11/15	
$C3H/HeN_X$	C3H/HeN	18/19	<0.001
$C3H/HeN_X$	C3H/HeJ	5/29	

[a] Subscript X denotes recipient animals that received 850 R of X-irradiation before reconstitution with 10^7 bone marrow cells.

[b] Necrotic response determined on day 3 after LPS (50 μg) injection.

[c] Data combined from two separate experiments.

TABLE 2. *Susceptibility of a C3H/HeJ-derived fibrosarcoma to LPS-induced necrosis when implanted in C3H/HeN, C3H/HeJ, and chimeric mice*

Recipient[a]	Source of bone marrow for reconstitution	Mice with necrotic tumors/ total mice injected[b]	P
C3H/HeJ		0/15	
$C3H/HeJ_X$	C3H/HeJ	2/10	<0.004
$C3H/HeJ_X$	C3H/HeN	6/6	
C3H/HeN		10/11	
$C3H/HeN_X$	C3H/HeN	3/4	<0.09
$C3H/HeN_X$	C3H/HeJ	2/10	

[a] Subscript X denotes recipient animals that received 850 R of X-irradiation before reconstitution with 10^7 bone marrow cells.

[b] Injected with 50 μg of LPS.

nificant changes in the in vivo response of tumor-bearing mice to the necrotizing effect of endotoxin. These data demonstrate that the tumor itself does not need to be LPS sensitive to be necrosed, which is strong evidence against a direct necrotic effect of LPS on the tumor. In addition, since it is extremely unlikely that the process of X-irradiation and bone marrow reconstitution would significantly alter the endothelial cells of the host, our findings argue against a direct effect of LPS on the tumor vasculature. Finally, since there is no evidence for any defect in LPS-induced complement activation in C3H/HeJ mice, it is unlikely that LPS-induced tumor necrosis is mediated via the complement system. Therefore, the finding that adoptive transfer of bone marrow cells transfers the ability of donor cells to mediate tumor necrosis strongly suggests that LPS acts on some bone marrow-derived cell type to induce tumor necrosis. The precise cell(s) and its mechanism of action remain unclear. However, the data of Carswell et al. (7) indicate that after BCG infection and endotoxin challenge macrophages release a soluble tumor necrosis factor that exerts a cytotoxic effect on tumor cells in vivo and in vitro. Macrophages can also be activated by LPS to kill tumor cells in vitro by a mechanism that requires cell-cell contact (15). Thus, there exist at least two pathways by which LPS can initiate macrophage-mediated tumor necrosis. In addition, it has recently been reported that LPS-mediated tumor graft rejection requires a radiosensitive lymphoid cell (3). Therefore, it is not yet clear whether other macrophage-related mechanisms and/or lymphoid cells are involved. However, our data strongly suggest that there is an essential requirement for lymphoreticular cells in the initiation of LPS-induced tumor necrosis.

We have now demonstrated that C3H/HeJ mice can be rendered sensitive to a number of diverse LPS-induced events including lethality (9) (Michalek et al., manuscript in preparation), production of serum amyloid-associated protein (14), interferon production (Michalek et al., manuscript in preparation), and tumor necrosis by the adoptive transfer of LPS-sensitive bone marrow cells. In addition, C3H/HeN mice can also be rendered resistant to all these effects by the adoptive transfer of LPS-resistant bone marrow cells. Thus, there is ample evidence to support the notion that the diverse manifestations of endotoxin reactions in the susceptible host require the participation of endotoxin-sensitive lymphoreticular cells and presumably a number of soluble mediators derived from such cells.

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