Radioprotection of LPS-resistant C3H/HeJ mice by RES-blockade

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Summary. The radioprotective effect of RES-blockade was studied in LPS-resistant mice. Injection of carbon particles protected these mice from radiation lethality whereas LPS did not; thus a difference in the mechanism of radioprotection was demonstrated.

Blockade of the reticuloendothelial system (RES) with a particulate substance enhances the restoration of hemopoietic cells after irradiation, resulting in increased survival of the animals[2-4]. Although the effect is similar to that of bacterial lipopolysaccharide (LPS), some differences have been suggested in the mechanism of radioprotection[5,6]. The C3H/HeJ mouse has a genetic resistance to a number of effects of LPS[7,8]. Furthermore, LPS has no radioprotective effect on these mice[9], which therefore provide a suitable model for study of the possible differences between the effect of RES-blockade and that of LPS on radiation lethality.

Materials and methods. Mice of C3H/HeJ or C3H/HeN strain were injected i.v. either with 8 mg carbon particles to blockade RES or with 5 µg LPS (*Salmonella typhosa* lipopolysaccharide 0901 (B), Difco, USA) at 9-10 weeks of age. 24 h later, they were given whole-body irradiation with X-rays operating at 180 kVp-20 mA with a filter of 1.0 mm Al + 0.5 mm Cu at a dose rate of 50 rads/min. The number of endogenous spleen colonies was assayed 9 days after treatment with 600 rads. Spleens were excised under ether anesthesia, weighed and fixed in Bouin's solution, and the colonies in the spleen were counted under a dissecting microscope.

Results and discussion. Pretreatment with carbon particles, or with LPS, protected LPS-sensitive C3H/HeN mice from the lethal effect of irradiation. However, C3H/HeJ mice were not protected by the pretreatment with LPS, but injection of carbon particles did protect even these LPS-resistant mice efficiently (table 1).

As shown in table 2, both strains of mice showed a significant increase in the number of endogenous spleen colonies after irradiation in response to the treatment with carbon particles or LPS. The spleen was somewhat enlarged in treated C3H/HeN mice but not in C3H/HeJ.

The effect of RES-blockade by carbon particles has been reported to be very similar to that of LPS-treatment[5]. In a previous paper, however, we have shown that radioprotection by RES-blockade is dependent on the elevated survival of hemopoietic stem cells (CFUs), whereas LPS can protect mice even when there is no increase in the survival of CFUs[6]. Present results demonstrate clearly that RES-blockade can protect LPS-resistant mice, indicating a difference in the mechanism of radioprotection between RES-blockade and LPS-treatment.

Another important finding is that LPS did not protect C3H/HeJ mice although it increased the survival of CFUs as much as carbon particles did. Although the survival and recovery of hemopoietic stem cells is mainly responsible for the survival of sublethally irradiated animals, the actual survival of the animals must ultimately depend on the restoration of the differentiated functional blood cells within a critical time after irradiation. It has been shown that LPS does not stimulate hemopoiesis, for example granulophilia, in resistant C3H/HeJ mice[8]. Thus, present findings suggest that not only the survival but also the unaltered differentiation of the hemopoietic stem cells is essential for the protection of the irradiated animals, and that RES-blockade provides a favorable environment for both the survival and the 'normal' differentiation of the stem cells. Since macrophages are the target cells for both the carbon particles and LPS[2,6], the difference may result from differences in the response of the phagocytic cells to LPS and 'nontoxic' carbon particles.

Table 1. Survival of mice treated either with carbon particles or with bacterial lipopolysaccharide (LPS), after irradiation

Treatment	C3H/HeN (700 rad)	C3H/HeJ (670 rad)
Control*	0/10 (0%)	0/10 (0%)
Carbon	10/10 (100%)	10/10 (100%)
LPS	10/10 (100%)	1/10 (10%)

Mice were injected i.v. with 8 mg carbon particles or with 5 µg LPS 24 h before irradiation. Survival was determined 30 days after irradiation. *Untreated control.

Table 2. Effect of carbon particles or LPS-treatment on endogenous spleen colony formation

Strain	C3H/HeN			C3H/HeJ		
Treatment	Control	Carbon	LPS	Control	Carbon	LPS
Spleen weight (mg)	42 ± 11	60 ± 10	94 ± 14	41 ± 9	45 ± 7	51 ± 4
No. of spleen colonies	2.7 ± 1.4	21.4 ± 5.0	numerous (> 30)	5.0 ± 1.6	19.7 ± 2.5	22.2 ± 5.6

Mice were injected with 8 mg carbon particles or with 5 µg LPS 24 h prior to 600 rad or irradiation. Spleen weight and number of spleen colonies were assayed 9 days later. 5-10 mice per group.

1 This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

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