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Ability of Post-Endotoxin Serum from BCG-Infected Mice to Induce Nonspecific Resistance and Stimulation of Granulopoiesis

RENATE URBASCHEK* AND BERNHARD URBASCHEK

Department of Immunology and Serology, Institute of Hygiene and Medical Microbiology, Klinikum Mannheim, University of Heidelberg, D65, 68 Mannheim, West Germany

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Serum from BCG-infected mice obtained 2 h after injection with endotoxin induced elevated levels of colony-stimulating factor and an increase in splenic granulocyte-macrophage progenitor cells in C3H/HeJ mice. The capacity of such serum to stimulate granulopoiesis may be related to its ability to increase nonspecific resistance to lethal irradiation.

Infection with *Mycobacterium bovis* BCG results in an increased nonspecific resistance to infection (6, 7) and, at the same time, in an increased susceptibility to endotoxin (ET) (11). Although the state of the activated macrophages is thought to be involved in the underlaying mechanisms, both effects can be achieved upon passive transfer of serum from BCG-infected mice after injection with ET (BCG-ET serum). Parant et al. (8) have demonstrated that such serum enhances resistance to bacterial infections, and it has been shown that BCG-ET serum induces increased susceptibility to ET in C3H/HeJ mice (14).

We were interested in determining whether BCG-ET serum enhances nonspecific resistance to irradiation and is capable of inducing granulopoiesis stimulation. Serum colony-stimulating factor (CSF, a factor required in vitro for the clonal proliferation of granulocyte-macrophage progenitor cells, CFU culture [CFUc]), was determined, as well as the splenic CFUc. To exclude effects due to the ET content in this serum, lipopolysaccharide-low responder C3H/HeJ mice (10) were used. These mice cannot be protected against irradiation death by ET (12) and do not respond to ET with elevated CSF or splenic CFUc (2).

Eight- to ten-week-old female C3H/HeJ and C3HeB/FeJ mice (Jackson Laboratories, Bar Harbor, Maine) and NMRI mice (Versuchstierzucht, Hannover, Germany) were used. NMRI mice were intravenously (i.v.) injected with 5×10^7 viable BCG organisms (*M. bovis* 1029 Phipps, lot A-17; Trudeau Institute, Saranac, N.Y.). Fourteen days later, they received 5 μg of ET prepared from *Escherichia coli* 0111 by the Boivin procedure. Two hours later, they were bled, and BCG-ET serum was pooled and stored at −80°C. In addition, mouse sera collected 14 days after BCG infection (BCG serum) and 2 h after i.v. injection of 5 μg of ET (ET serum) and serum from nontreated mice (control serum) were used.

Ten C3H/HeJ mice per group were injected intraperitoneally and i.v. with 0.25 ml of these sera 24 h before or 48 h after irradiation. Control groups were injected with saline. The mice were subjected to 580 rads whole-body X-irradiation in a rotating circular plastic holder at 200 kV and 20 mA (filtration, 0.5 mm of Cu; focus and target distance, 8 cm; dosage rate, 69 rads/min).

BCG-ET serum induced marked radioprotection in C3H/HeJ mice when given 24 h before whole-body X-irradiation (Fig. 1). Similar protection was achieved when BCG-ET serum was injected 48 h after irradiation; and 80% lethal dose within 30 days postirradiation was reduced to a 14% lethal dose within 30 days postirradiation. ET serum did not decrease the lethality rate after X-irradiation exposure in C3H/HeJ mice. Thus, it is apparent that a humoral factor(s) in this BCG-ET serum is responsible for the enhanced nonspecific resistance. Such a role has been discussed for CSF, which is also elevated after BCG infection (4) and in such sera (13). However, it has been reported that, upon passive transfer, purified CSF failed to protect mice against irradiation (13); the extent of radioprotection did not correlate with the CSF content of post-ET serum or its fractions from Zymosan-treated mice (1). This does not exclude the essentiality of CSF, since the content of CSF in the transferred serum might be less important than the capacity of this serum or its fractions to induce elevated serum endogenous CSF and an increase in splenic CFUc. As a result, the following experiment was performed.

Serum CSF (2 h after serum transfer) and the number of splenic CFUc (3 days after serum

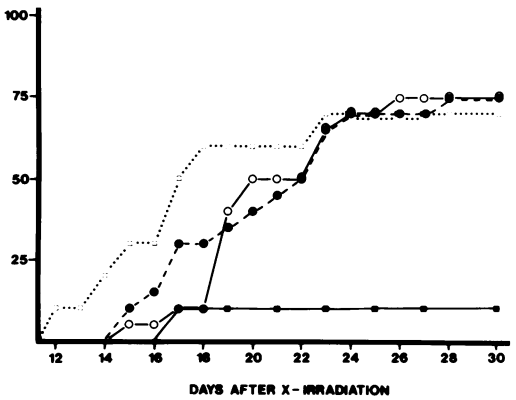

FIG. 1. Percent cumulative lethality of C3H/HeJ mice injected 24 h before whole-body X-irradiation (570 rads) with normal serum (●), serum obtained 2 h after i.v. injection with 5 μg of ET (□), or 2 h after i.v. injection with 5 μg of endotoxin from BCG-infected mice (■). The control group was injected with saline (○).

transfer) were assayed by the semi-solid agar technique (3, 9). To determine serum CSF, triplicate cultures were plated with 25 μl of serum from five mice each per group per 10^5 nucleated femoral cells. Aggregates of greater than 50 cells were scored as colonies after 7 days of incubation at 37°C in a humidified atmosphere containing 7.5% CO_2. The number of splenic CFUc was obtained correspondingly with 25 μl of post-ET serum per plate as source of standard CSF. Each culture contained 10^6 nucleated splenic cells.

Table 1 demonstrates that BCG-ET serum elicited an increase in serum CSF in C3H/HeJ mice 2 h after passive transfer. BCG-ET serum also led to a marked increase in the number of splenic CFUc in C3H/HeJ mice (Table 2). As was expected, C3H/HeJ mice did not respond to the transfer of ET serum. In contrast, the sera of

TABLE 1. Serum CSF in C3H/HeJ and C3HeB/FeJ mice 2 h after serum transfer

Serum[a]	Serum CSF ± SD[b]	
	C3HeB/FeJ	C3H/HeJ
Control	0	0
BCG	0	0.67 ± 1.15
ET	75.80 ± 6.90	0.53 ± 0.84
BCG-ET	71.53 ± 2.50	65.33 ± 5.57

[a] Sera obtained from untreated NMRI mice (control serum), 14 days after BCG infection (BCG serum), 2 h after i.v. injection with 5 μg of ET (ET serum), or 2 h after i.v. injection with 5 μg of ET from BCG-infected mice (BCG-ET serum).

[b] Expressed as number of colonies per 10^5 nucleated bone marrow cells, using 25 μl of serum.

TABLE 2. Splenic CFUc in C3H/HeJ and NMRI mice 3 days after serum transfer

Serum[a]	No. of CFUc ± SD per spleen[b]	
	NMRI	C3H/HeJ
Saline	8,000 ± 323	10,400 ± 976
Control	9,410 ± 526	10,900 ± 529
BCG	16,000 ± 947	8,260 ± 609
ET	23,200 ± 1,420	6,570 ± 273
BCG-ET	30,400 ± 1,980	29,300 ± 3,880

[a] Sera obtained from untreated NMRI mice (control serum), 14 days after BCG infection (BCG serum), 2 h after i.v. injection with 5 μg of ET (ET serum), or 2 h after i.v. injection with 5 μg of ET from BCG-infected mice (BCG-ET serum).

[b] The number of CFUc per spleen was calculated from the number of colonies per 10^6 splenic nucleated cells, using 25 μl of CSF-standard serum.

C3HeB/FeJ mice contained elevated levels of CSF, and the spleens of NMRI mice contained increased numbers of CFUc for which the endotoxin content could have been responsible. As determined with a quantitative turbidometric microtiter *Limulus* amebocyte lysate assay (5), sera obtained 2 h after injecting 5 μg of endotoxin i.v. contained 380 ng of ET per ml, and BCG-ET serum contained 140 ng of ET per ml (14). In earlier studies, we observed that the injection of 10 ng of endotoxin resulted in a CSF level of 54 ± 3 colonies per 10^5 bone marrow cells in C3HeB/FeJ mice.

These results clearly indicate that passive transfer of BCG-ET serum is capable of inducing a distinct stimulation of granulopoiesis. Since ET in such serum is not responsible for this effect, the serum contains a factor(s) that mediates elevation of CSF and CFUc which may be essential for the induction of increased resistance to irradiation and bacterial infection.

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