

Host Resistance to *Listeria monocytogenes* Infection Is Enhanced but Resistance to *Staphylococcus aureus* Infection Is Reduced in Acute Graft-versus-Host Disease in Mice

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Acute graft-versus-host disease (GVHD) is characterized by the production of high levels of T helper 1 (Th1)-type cytokines. Bone marrow transplantation from allogeneic C57BL/6 cells to CBF₁ mice produced acute GVHD. Host resistance to Th1-driven *Listeria monocytogenes* was enhanced, whereas host resistance to Th2-driven *Staphylococcus aureus* was reduced during acute GVHD. These results suggest that opposite host responses are observed between Th1-driven and Th2-driven bacterial infections in acute GVHD.

Acute graft-versus-host disease (GVHD) is the most common complication of allogeneic bone marrow transplantation (BMT). During acute GVHD, cytokine dysregulation occurs as a consequence of synergistic interactions between cells of both myeloid and lymphoid lineages (14). Antigen-specific CD4⁺ T helper (Th) cell responses can be divided into two types, Th1 and Th2, based on cytokine production and effector function (15, 28). Differentiation of Th1 cells, which can produce interleukin 2 (IL-2), gamma interferon (IFN- γ), and lymphotoxin, is driven by IL-12 and IFN- γ , while differentiation of Th2 cells, which can produce IL-4, IL-5, IL-10, and IL-13, is driven by IL-4. Acute GVHD is characterized by the production of high levels of Th1-type cytokines, including IFN- γ (1, 23). The perceived role of IFN- γ in acute GVHD has been conflicting. Some studies have demonstrated that IFN- γ plays an important role in the pathogenesis in acute GVHD (4, 32), and other studies have suggested that IFN- γ plays a role in protecting from the disease (16, 33).

Listeria monocytogenes, a facultative intracellular bacterium, induces the Th1 response, including IFN- γ production in the infected host (11), and IFN- γ plays a critical role in antilisterial resistance (8, 12), while Th2-type cytokines, including IL-4 and IL-10, inhibit antilisterial resistance (3, 17, 18, 31). In contrast, our recent study indicated that Th2 response becomes dominant in *Staphylococcus aureus* infection and that IL-4 and IL-10 might play a protective role (25) but that IFN- γ plays a detrimental role in host resistance against *S. aureus* infection (19, 25, 34). On the other hand, infections of these pathogens have been reported as the complication of GVHD in humans (6, 26, 35). Therefore, we were interested in investigating the host response to *L. monocytogenes* or *S. aureus* infection in a mouse model of acute GVHD which is polarized toward Th1.

In this study, data were expressed as means \pm standard deviations (SD), and the Wilcoxon rank-sum test was used to determine the significance of the differences in bacterial counts in the organs and in cytokine titers between the control and experimental groups. The generalized Wilcoxon test was used

to determine the significance of differences in the survival rates.

Female C57BL/6 mice (B6 mice; H-2^b) and CBF₁ mice (BALB/c \times B6 H-2^{b \times d}), 6 to 8 weeks old, were purchased from SLC Japan, Hamamatsu, Shizuoka, Japan. All animals were maintained in the Institute for Animal Experiment, Hirosaki University School of Medicine. Acute GVHD was induced in irradiated CBF₁ mice by intravenous injections of bone marrow cells and spleen cells of B6 mice as described previously (9, 30). Briefly, CBF₁ mice received a sublethal 8.0-Gy dose of total body irradiation from an X-ray irradiation apparatus (MBR-1505R2; Hitachi Medico, Ibaraki, Japan). The recipients were injected intravenously with 1×10^7 bone marrow cells and 5×10^7 spleen cells of B6 mice resuspended in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) 24 h later. As a control, irradiated CBF₁ mice were injected with 1×10^7 bone marrow cells and 5×10^7 spleen cells of CBF₁ mice. B6 BMT recipients started to lose body weight from day 5 of transplantation as a typical clinical sign of acute GVHD (Fig. 1A). Furthermore, most B6 BMT recipients had diarrhea and lost hair during observation.

Endogenous cytokines in the spleen and liver homogenates and cytokine responses in the spleen cell cultures of B6 BMT recipients and CBF₁ BMT control recipients were investigated on day 7 of transplantation. Preparation of spleen extracts for cytokine assays and preparation of splenocytes for in vitro cultures were performed as described previously (20, 24). Assays for IFN- γ , IL-4, and tumor necrosis factor (TNF- α) were carried out by a double sandwich enzyme-linked immunosorbent assay, as described previously (18, 19). The endogenous IFN- γ titers were significantly higher in the spleens and livers of B6 BMT recipients, compared with those of CBF₁ BMT control recipients (Fig. 1B). The higher titers of TNF- α , which is involved in the pathogenesis in acute GVHD (29), were also detected in B6 BMT recipients (Fig. 1C). Next, splenocytes (10^6 /ml), which were isolated from B6 BMT recipients or CBF₁ BMT control recipients on day 7 after transplantation, were inoculated into a 24-well tissue culture plate (Greiner, Frickhausen, Germany), and the cells were stimulated with 2.5×10^6 cells of irradiated splenocytes obtained from B6 mice or from CBF₁ mice or stimulated with 145-2C11-derived anti-mouse CD3 ϵ monoclonal antibody at a final concentration of 2 μ g/ml at 37°C in a humidified 5% CO₂ incubator for 48 h.

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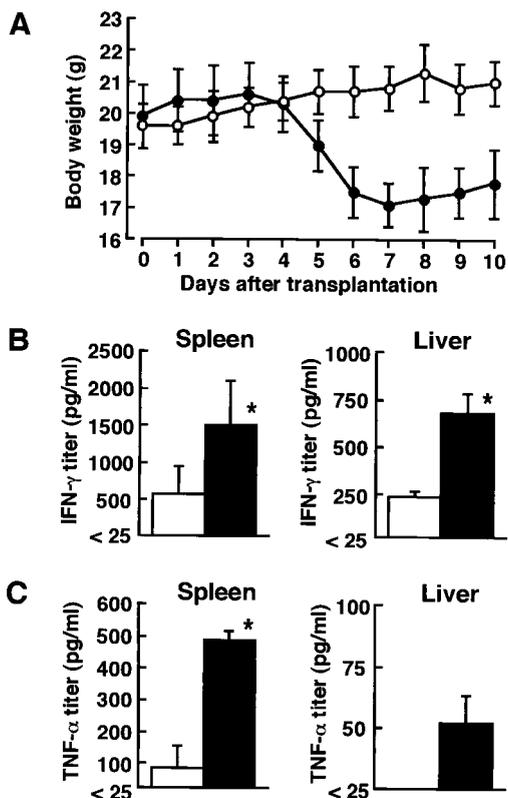


FIG. 1. (A) Effect of allogeneic BMT on body weight and endogenous cytokine production. Body weights of B6 BMT recipients (solid circles) and CBF₁ BMT recipients (open circles) were expressed the means ± SD (error bars) for a group of six mice. The data are a representative result of five experiments. (B and C) The titers of endogenous IFN-γ (B) and TNF-α (C) in the spleens and livers of B6 BMT (solid bars) and CBF₁ BMT (open bars) recipients were assayed. Each result represents the mean ± SD for a group of four mice. These results were reproduced in three repeated experiments. A single asterisk indicates a significant difference from the CBF₁ BMT recipients at a P value of <0.01.

Culture supernatant fluids obtained were assayed for IFN-γ and IL-4 (Table 1). Splenocytes obtained from B6 BMT recipients produced IFN-γ but not IL-4 in response to irradiated CBF₁ splenocytes, and both cytokines were produced in response to stimulation with anti-CD3 monoclonal antibody. They did not respond to syngeneic B6 splenocytes. In contrast,

TABLE 1. Cytokine production in spleen cell cultures obtained from mice which received allogeneic or syngeneic BMT^a

Mice	Stimulation	Titer (pg/ml) of:	
		IFN-γ	IL-4
CBF ₁ →CBF ₁	B6 splenocytes	<25	<25
	CBF ₁ splenocytes	<25	<25
	Anti-CD3	<25	<25
B6→CBF ₁	B6 splenocytes	<25	<25
	CBF ₁ splenocytes	1,108 ± 188	<25
	Anti-CD3	4,030 ± 1493	60 ± 5

^a Spleen cells obtained from CBF₁ mice, which had been transplanted with bone marrow cells and spleen cells obtained from B6 mice (B6→CBF₁) or CBF₁ mice (CBF₁→CBF₁) 7 days before were stimulated with irradiated B6 or CBF₁ splenocytes or with anti-CD3 monoclonal antibody for 48 h, and then the titers of IFN-γ and IL-4 in the culture supernatants were determined. Each result represents the mean ± SD for a group of five mice. The data are reproduced in three experiments.

TABLE 2. Effect of allogeneic BMT on host resistance against *L. monocytogenes* infection and *S. aureus* infection

Infection ^a	Days postinfection ^b	Mouse group ^c	Log bacteria (CFU/organ) ^d in:		
			Spleen	Liver	Kidney
<i>L. monocytogenes</i>	2	CBF ₁ →CBF ₁	4.81 ± 0.18	4.13 ± 0.26	ND ^e
		B6→CBF ₁	<2	<2	ND
	4	CBF ₁ →CBF ₁	4.14 ± 0.17	2.60 ± 0.44	ND
		B6→CBF ₁	<2	<2	ND
<i>S. aureus</i>	2	CBF ₁ →CBF ₁	2.08 ± 0.36	4.26 ± 0.06	6.35 ± 0.67
		B6→CBF ₁	3.51 ± 0.46 ^f	4.26 ± 0.13	6.63 ± 0.45
	7	CBF ₁ →CBF ₁	2.25 ± 0.19	3.29 ± 0.26	7.26 ± 0.65
		B6→CBF ₁	3.95 ± 0.37 ^f	5.79 ± 0.26 ^f	7.68 ± 0.34

^a BMT recipients were infected intravenously with 10⁴ CFU of *L. monocytogenes* or 10⁷ CFU of *S. aureus* on day 7 post-BMT.
^b The number of bacterial cells in the organs was determined at the indicated times.
^c Irradiated CBF₁ mice were transplanted with bone marrow cells and spleen cells obtained from B6 mice (B6→CBF₁) or CBF₁ mice (CBF₁→CBF₁).
^d Each result represents the mean ± SD for a group of four mice. These results were reproduced in three repeated experiments.
^e ND, not determined.
^f Significantly different from value for CBF₁→CBF₁ group (P < 0.05).

splenocytes obtained from CBF₁ BMT recipients failed to produce these cytokines in response to any stimuli used herein. These results suggest that the production of the Th1-type cytokine IFN-γ is augmented in B6 BMT recipients.

The effect of acute GVHD on host resistance against *L. monocytogenes* infection was investigated. *L. monocytogenes* 1b-1684 cells were prepared as described previously (18). B6 BMT recipients and CBF₁ BMT control recipients were infected intravenously with 10⁴ CFU of *L. monocytogenes* on day 7 post-BMT, and the number of bacterial cells in the spleens and livers was determined on days 2 and 4 of infection by culturing on tryptic soy agar (Difco Laboratories, Detroit, Mich.) (18) (Table 2). Bacteria in the organs were not detectable on days 2 and 4 of infection in B6 BMT recipients, while significant numbers of bacteria in the organs were observed on day 2 and then the numbers were decreased on day 4 of infection in CBF₁ BMT control recipients. It is recognized that antilisterial resistance depends on innate immunity on day 2 and is performed by cytokine-induced activated phagocytes on day 4 of systemic *L. monocytogenes* infection (13). Therefore, the results suggest that innate immunity to *L. monocytogenes* infection is augmented in acute GVHD.

Next, the effect of acute GVHD on host resistance to *S. aureus* infection was investigated. *S. aureus* 834 cells were prepared as described previously (24). B6 BMT recipients and CBF₁ BMT control recipients were infected intravenously with 10⁷ CFU of *S. aureus* on day 7 post-BMT. Half of the infected B6 BMT recipients succumbed until day 6 of infection, whereas all CBF₁ BMT control recipients survived (Fig. 2). The number of bacterial cells in the spleens, livers, and kidneys of the infected mice was determined on days 2 and 7 of infection (Table 2). It is presumed that host resistance depends on innate immunity on day 2, while the reduction of bacterial numbers is observed by day 7 in the spleens and livers during systemic *S. aureus* infection (19, 25). Bacterial numbers in the spleens on day 2 and in the spleens and livers on day 7 but not in the kidneys were markedly increased in B6 BMT recipients, compared with those in preferentially CBF₁ BMT control recipients. Our previous study indicated that *S. aureus* colonizes and proliferates along with abscess formation in kidneys, but large numbers of bacterial cells were detected in spleen and liver when they were shifted to a lethal systemic infection (19).

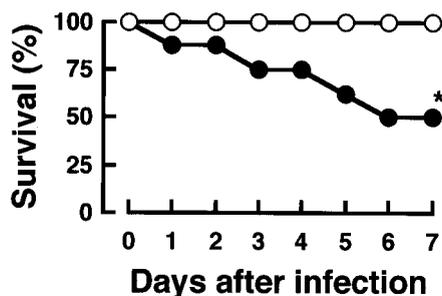


FIG. 2. Effect of allogeneic BMT on survival rates of mice infected with *S. aureus*. Eight B6 BMT recipients (solid circles) and eight CBF₁ BMT control recipients (open circles) were infected intravenously with 10^7 CFU of *S. aureus* on day 7 post-BMT. An asterisk indicates a significant difference from the CBF₁ BMT recipients at a *P* value of <0.01. These results were reproduced in three repeated experiments.

Therefore, it is possible that B6 BMT recipients might succumb to septicemia. The present results suggest that B6 BMT recipients are more susceptible to *S. aureus* infection during acute GVHD.

In the present study, opposite susceptibilities between Th1-driven and Th2-driven bacterial infections were expressed in acute GVHD. A Th1-driven acute GVH reaction augmented host resistance to Th1-driven *L. monocytogenes* infection in the early stage (Table 2). It is known that Th1 or Th1-related cytokines, especially IFN- γ and TNF- α , are involved in anti-listerial resistance (8, 10, 12, 17, 21, 22). Therefore, in the present study, upregulation of innate immunity to *L. monocytogenes* infection might be due to activation of effector cells, including macrophages (2, 13), by cytokines, including IFN- γ and TNF- α produced during acute GVH reaction (Fig. 1 and Table 1). Recently, some studies showed that prior or concurrent immunization with bacteria such as *Mycobacterium bovis* BCG and *Brucella abortus*, which induce Th1-type responses, abrogates antigen-specific, immunoglobulin E-mediated allergic recall response to ovalbumin (5, 27). Similarly, our present results suggested that host resistance to *S. aureus* infection, in which IL-4 and IL-10 might play a beneficial role and IFN- γ might play a detrimental role (19, 25), was suppressed during Th1-type acute GVHD (Fig. 2 and Table 2). In humans, staphylococci are involved in opportunistic infections in patients with GVHD (6, 26). Given our present results, it may be significant that *S. aureus*, which causes nosocomial infections, is noticed in acute GVHD.

In conclusion, opposite susceptibilities to infectious diseases may be observed in BMT recipients suffering from acute GVHD, depending on the inducibility of Th cell responses by pathogens.

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