Alloimmune and Skin Allograft Responses in 4-1BB (CD137)-deficient Mice

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ABSTRACT

Background: The costimulatory molecule 4-1BB, a member of nerve growth factor receptor/tumor necrosis factor (NGFR/TNFR) super family, is involved in cell survival and death. **Methods:** In this study, female C57BL/6 (H-2^b) mice were used as a recipient, and DBA/2 (H-2^d) as a donor to assess a mixed lymphocyte reaction (MLR) and CTL response in vitro, and skin graft survival. IL-2, IFN level was measured by ELISA. **Results:** Mixed lymphocyte reaction (MLR) analysis showed that 4-1BB-deficient responder cells showed enhanced cellular proliferation over littermate controls. In contrast, IL-2 production was diminished only in 4-1BB knockout cultures. The IFN expression, on the other hand, was comparable between the groups. When female C57BL/6 (H-2^b) mice were grafted with the trunk skin of DBA/2 (H-2^d) mice, the in vivo tissue destruction of 4-1BB-deficient mice was not distinct from the normal littermates. **Conclusion:** These data suggest that 4-1BB is critical for the induction of alloreactive responses in vitro but 4-1BB alone could not change the course of skin rejection in vivo. **(Immune Network 2002;2(3):133-136)**

Key Words: 4-1BB, skin graft, alloimmunity, MLR

Introduction

The inducible T cell antigen 4-1BB is a member of NGFR/TNFR superfamily and is expressed on the surface of CD4+ and CD8+ T cells (1-5). Others and we have shown that signals relayed through the murine T cell antigen 4-1BB enhance primary T cell responses, and that blocking the interaction of 4-1BB with its ligand results in decreased responses to polyclonal activation and to alloantigens (1,6-9). 4-1 BB is coupled to tyrosine kinase, $p56^{lck}$, suggesting that kinase may play a role in transmitting signals delivered through 4-1BB (10). 4-1BB binds a high affinity ligand (4-1BBL) on activated APCs, to transmit a distinct and potent costimulatory signal through the TRAF2-NIK pathway and activates NF-KB (11,12). The signals mediated by 4-1BB are distinct from those of CD28 and results in a greater spectrum of cytokines than the CD28 signaling pathway (1,8,11,13 -15). Antibodies to the 4-1BB have been shown to increase graft versus host disease (GVHD), accelerate the rejections of cardiac and skin allografts, and eradicate established tumors, and prevent activation-induced cell death (AICD) (8,9,16-18).

Recent studies reported that signals delivered through 4-1BB preferentially induce CD8+ T cell proliferation and demonstrate that anti-4-1BB mAbs enhanced the rapidity of skin transplant rejection in mice (9). In contrast, our data on the characterization of 4-1BB-deficient mice suggested that these mice have dysregulated cellular proliferation and plays an important role, among others, in myelopoiesis (19). To further assess the importance of 4-1BB in immunity, in the present study we have examined the allogeneic response in 4-1BB-deficient mice. The study revealed that the costimulatory signals generated through 4-1BB are not critical for the initial activation and amplification of T cells in MLR, but 4-1BB appears to play a significant role in cytokine production and enhancement of CTL alloreactivity in vitro.

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However, the role of 4-1BB in modulating in vivo tissue destruction is not so distinct.

Materials and Methods

Mice. DBA/2 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). 4-1BB-deficient mice were generated by gene targeting in our laboratory and have been back crossed more than 8 times to C57BL/6 as described earlier (19) and maintained in animal facility under the specific pathogen-free conditions at the Immunomodulation Research Center, University of Ulsan. Mice heterozygous for 4-1BB gene mutation and wild types were used as controls.
Mixed leukocyte reaction (MLR). The activities of splenic T cells were evaluated by culturing responder spleen cells with 20 Gy-irradiated stimulator spleen cells (DBA/2) for allogeneic MLR. The cells were mixed in RPMI-1640 medium at a concentration of 1×10^6 of both responder and stimulator cells. A total volume of 200μl/well for triplicate cultures in a 96-well microtiter plate were incubated at 37°C and 5% CO2 in a humidified atmosphere. On days 2, 3, or 4 of incubation, the cultures were pulsed with 1.0μci/well [^{3}H]-thymidine. The cells were harvested 15-20 h later and the incorporated radioactivity was counted in a liquid scintillation and expressed as counts per minute (CPM).
Cytokine ELISA. Supernatants (100μl/well) from MLR were harvested at 24 and 48 hr incubation and assayed for the presence of IL-2 and IFN by ELISA.
Cytotoxicity assay. Alloreactive CTL were induced in 5-day MLR by incubating 25×10^6 responder spleen cells with an equal number of irradiated (20 Gy) allogeneic spleen cells in 20 ml medium in tissue culture flask kept in upright position. Cytotoxic activity of the recovered cells was determined as described by Watanabe et al (20). Briefly, CTL alloreactivity assessed by 4 h ^{51}Cr released assay using 2-day-old ConA-induced lymphoblasts as target cells. The percentage of cell lysis was calculated as [(experimental release-spontaneous release/maximum spontaneous release)]×100.
Skin grafting. Full-thickness trunk skin pieces (10×10 mm) from DBA/2 donor mice were grafted onto the dorsal thorax of C57BL/6 recipient mice as described by Kawai, et al (21). Dressings were removed 8 days after transplantation and grafts were scored daily until rejection. Grafts were defined as rejected when complete loss of the intact epithelia occurred. Graft survival data were analyzed for significance using the turkey multiple comparison test.

Results and Discussion

4-1BB-deficient responder cells display enhanced MLR. Given our recent observation that 4-1BB-deficient T cells exhibited dysregulated cellular proliferation to polyclonal activators (19), we wanted to know if the same is displayed in an MLR set up. To examine our hypothesis, responder T cells from 4-1BB (H-2^b)$^{+/+}$, 4-1BB (H-2^b)$^{+/-}$ or 4-1BB (H-2^b)$^{-/-}$ mice were cultured with allogeneic DBA/2 (H-2^d) stimulator cells. The result depicted in Fig. 1 showed that 4-1BB-deficient T cells have significantly higher ($P<0.01$) proliferative response on day 3, 4, and 5 of MLR compared to the wild type controls. The increased proliferation of lymphocytes in 4-1BB-deficient mice upon allogeneic stimulation suggests a role for this molecule in regulating cell growth. It is not clear as to why 4-1BB-deficiency unleashes enhanced cellular proliferation. Our recent studies suggested that 4-1 BBL might have an important role in this cellular dysregulation as introduction of 4-1BBL-bearing cells into the cultures abrogated the phenomenon (19). To further determine whether the enhanced proliferative response in 4-1BB-deficient mice was also accompanied by an increase in cytokine production, the MLR supernatants using irradiated DBA/2 stimulator cells were assayed for IL-2 and IFN production. The result in Fig. 2 showed an inverse correlation in the MLR cytokine production by 4-1BB-deficient mice. The IL-2 secretion was inhibited in 4-1BB- deficient mice but the IFN expression was marginally affected. This is in line with our recent data (19) that enhanced cellular proliferation in these mice is not dependent on IL-2.
Cytotoxic responses but not graft survival rates are reduced in 4-1BB-deficient mice. In allograft rejection experiments, cytotoxic activity of effector cells has been commonly used as a test of T lymphocytes to mediate in vivo

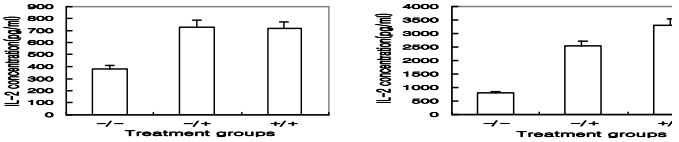

Figure 1. Enhanced T cell proliferative responses in 4-1BB-deficient mice. Responder T cells from C57BL/6 wild type control (triangle), heterozygous (square), and 4-1BB-deficient (circle) mice were cultured with irradiated stimulator cells from DBA/2 mice. The proliferation was measured by [^{3}H]-thymidine assay on the indicated days and expressed as CPM. Representative data from 6 independent experiments are shown. Each line represents mean CPM of progeny from 6 different litters (two progeny/litter). Vertical lines represent standard errors from the mean.

Figure 2. Reduced IL-2 but comparable IFNγ production by 4-1BB-deficier responder T cells from wild type control (4-1BB$^{+/+}$), heterozygous (4-1BB$^{+/-}$) to DBA/2 stimulator cells after 24 h activation. (B) IL-2 production by resp after 48-hr activation. (C) IFNγ production by responder T cells in response bar represents the mean cytokine production expressed in pg/ml secreted by progeny/litter). Representative data from 6 independent experiments are shown

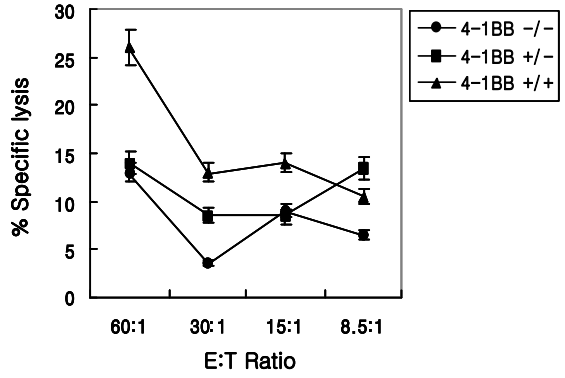

Figure 3. Reduced alloreactive cytotoxic activity in 4-1BB-deficient T cells. Responder T cells from C57BL/6 wild type control (triangles), heterozygous (squares), and 4-1BB-deficient (circles) mice were cultured with irradiated DBA/2 stimulator cells for 5 days. Effector cells were recovered and added to ^{51}Cr-labeled ConA-induced lymphoblast target cells at various effector to target ratios for 4 h. Range of spontaneous ^{51}Cr release was between 10～15%. Representative data from 4 independent experiments are shown. Each line represents the mean percentage of specific target lysis by effector cells from progeny of 4 different litters (two progeny/litter). Vertical lines represent standard errors from the mean.

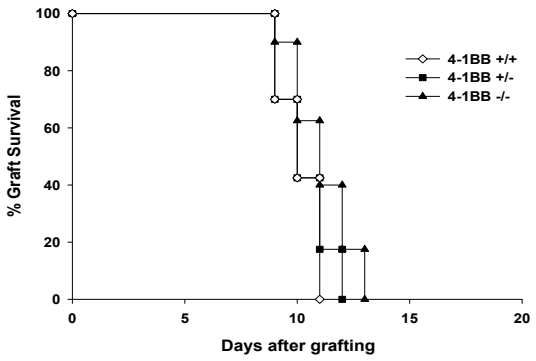

Figure 4. Skin allograft survival in 4-1BB-deficient mice is not altered. Wild type C57BL/6 control (triangles), heterozygous (squares), and 4-1BB-deficient (circles) mice received DBA/2 skin graft transplants. Representative data from 4 independent experiments using mice from 4 different litters (4 progeny/litter) are shown. Graft survival data were analyzed for significance using the Turkey multiple comparison test.

tissue destruction. To measure induction of alloreactive cytotoxic activity in 4-1BB-deficient mice, cytolytic T lymphocytes were induced in 5 day MLR. The cells were recovered and cytotoxic activity was determined by standard 4 h ^{51}Cr released assay using 2-day ConA-induced lymphoblasts as target cells. The result in Figure 3 showed that 4-1BB-deficient effector cells were defective in alloreactive cytotoxic responses compared to the wild type. This is in contrast to the observation that in MLR response, the 4-1BB-deficient mice showed enhanced cell proliferation.To determine the functional consequences of the 4-1BB costimulator pathway in vivo, the skin allograft was examined in 4-1BB-deficient mice. The result in Figure 4 showed that trunk skin graft from DBA/2 mice was readily rejected in 4-1BB-deficient C57BL6/ mice. Although there was no statistical significance in the graft survival time among 4-1BB$^{+/+}$, 4-1BB$^{+/-}$ and 4-1BB$^{-/-}$ mice, a prolonged graft survival was observed in 4-1BB-deficient mice as compared with the wild type control recipient mice. Since the rapidity of graft rejection is determined by many different factors, 4-1BB-deficiency alone might not result in significant prolongation of graft survival.

In conclusion, we showed that the costimulatory signals generated through 4-1BB are not critical for the initial activation and amplification of T cells in allogeneic MLR. Lymphocytes lacking 4-1BB exhibit enhanced cell proliferation rather than an orderly pathway towards differentiation. The costimulatory molecule 4-1BB appears to play a regulatory role in Th1 cytokine (such as IL-2 and IFNγ) production and enhanced CTL alloreactivity. Skin graft survival was comparable in 4-1BB-deficient and 4-1BB intact mice. This extension might be attributed to decreased alloreactive effector cells.

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