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Preferential Blockade of CD8^{+} T Cell Responses by Administration of Anti-CD137 Ligand Monoclonal Antibody Results in Differential Effect on Development of Murine Acute and Chronic Graft-Versus-Host Diseases[1]

Kazuhisa Nozawa,*[†¶] Junko Ohata,*[∥] Jinkyo Sakurai,*[∥] Hiroshi Hashimoto,[†] Hiroaki Miyajima,[‡] Hideo Yagita,[§] Ko Okumura,[§] and Miyuki Azuma[2]*[¶]

We investigated the effect of CD137 costimulatory blockade in the development of murine acute and chronic graft-vs-host diseases (GVHD). The administration of anti-CD137 ligand (anti-CD137L) mAb at the time of GVHD induction ameliorated the lethality of acute GVHD, but enhanced IgE and anti-dsDNA IgG autoantibody production in chronic GVHD. The anti-CD137L mAb treatment efficiently inhibited donor CD8^{+} T cell expansion and IFN-γ expression by CD8^{+} T cells in both GVHD models and CD8^{+} T cell-mediated cytotoxicity against host-alloantigen in acute GVHD. However, a clear inhibition of donor CD4^{+} T cell expansion and activation has not been observed. On the contrary, in chronic GVHD, the number of CD4^{+} T cells producing IL-4 was enhanced by anti-CD137L mAb treatment. This suggests that the reduction of CD8^{+} T cells producing IFN-γ promotes Th2 cell differentiation and may result in exacerbation of chronic GVHD. Our results highlight the effective inactivation of CD8^{+} T cells and the lesser effect on CD4^{+} T cell inactivation by CD137 blockade. Intervention of the CD137 costimulatory pathway may be beneficial for some selected diseases in which CD8^{+} T cells are major effector or pathogenic cells. Otherwise, a combinatorial approach will be required for intervention of CD4^{+} T cell function.   *The Journal of Immunology,* 2001, 167: 4981–4986.

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uccessful T cell activation requires engagement of the TCR with Ag/MHC as well as engagement of costimulatory molecules. The most extensively characterized costimulatory molecules between T cells and APC are CD28-B7 family molecules. Recently, a number of receptor and ligand pairs that belong to the TNF and TNF receptor families have been identified. CD137 ligand (CD137L,[3] 4-1BBL) and CD137 (4-1BB) is one of such pair (1, 2). CD137 is a type I transmembrane protein expressed on activated CD4^{+} and CD8^{+} T cells. CD137L is a type II surface glycoprotein expressed on APC such as activated B cells, macrophages, and dendritic cells (1–4). Ligation of CD137 in addition to TCR engagement costimulates T cell proliferation and IL-2 production (5). CD137 costimulatory signals preferentially induce proliferation, IFN-γ production, and survival by

CD8^{+} T cells in vitro (6, 7). The administration of anti-CD137 mAb or the transduction of CD137L into tumor cells efficiently amplifies antitumor CTL responses (8–10). In transplantation models the in vivo administration of mAb against CD137 enhances acute graft-vs-host disease (GVHD) by preferential amplification of CD8^{+} T cells and accelerates the rejection of cardiac allograft and skin transplants (6). The studies using CD137L-deficient mice also revealed the importance of CD137 costimulation in antiviral CTL responses (11–13). Thus, the accumulating reports suggest a crucial role of CD137 costimulation for CD8^{+} T cell responses. On the other hand, the influence of CD137 costimulation on CD4^{+} T cell responses has been shown, but in some limited situations and to a lesser extent (6, 14, 15). Although a regulatory effect of CD137 signal on CD28-mediated Th2 cell development (16) and a preferential high expression and functional contribution of CD137 by Th2 cells (17) have been reported, little is known about the actual contribution of CD137 to Th1- and Th2-meditaed immune responses in vivo.

A GVHD can be caused in inbred F$_1$ mice by the injection of T cells of parental origin. The injection of C57BL/6 (B6) splenocytes into F$_1$ mice (referred to as B6 GVH) results in an immunosuppressive acute GVHD (aGVHD) characterized by an anti-host cell-mediated, Th1 cytokine-driven disease (18–20). By contrast, the injection of splenocytes from the BALB/c or DBA/2 parent into F$_1$ mice (referred to as BALB/c GVH or DBA GVH) results in an immunostimulatory chronic GVHD (cGVHD) characterized by an autoantibody-mediated, Th2 cytokine-driven disease (21–23). In development of both types of GVHD, multiple costimulatory molecules are involved. It has been shown that blockade of either CD28 or CD40 signals inhibits both types of GVHD (24–28). In this study we have investigated the effects of CD137 blockade by using mAb against CD137L in both acute and chronic GVHD models and further examined donor cell expansion and effector function in both CD4^{+} and CD8^{+} T cells after the treatment.

Materials and Methods

Mice

Female C57BL/6 (B6, H-2^b), DBA/2 (H-2^d), (BALB/c × C57BL/6)F$_1$ (CBF1, H-2$^{b/d}$), and (C57BL/6 × DBA/2)F$_1$ (BDF1, H-2$^{b/d}$) mice were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan). Female C57BL/6 Ly5.1 (B6Ly5.1) mice were provided by Dr. H. Nakauchi (Tsukuba University, Tsukuba, Japan) and were bleed in our animal facility. Donors for bone marrow (BM) were 6 wk old, those for splenocytes were 6–12 wk old, and recipients were 8–9 wk old at the time of BM transplantation (BMT). For in vitro assay, female 6- to 8-wk-old BALB/c (H-2^d) and B6 mice were also obtained from SLC. Mice were maintained in a specific pathogen-free microisolator environment. All mice procedures were reviewed and approved by the animal care and use committee of the National Children's Medical Research Center and the Tokyo Medical and Dental University (Tokyo, Japan).

Monoclonal Abs and flow cytometry

Hybridomas producing anti-mouse CD80 (RM80, rat IgG2a), CD86 (PO3, rat IgG2b), and CD137L (TKS1, rat IgG2a) mAbs were generated and purified from ascites as described previously (29–31). The pyrogen level was <0.01 ng/ml protein, as determined by a *Limulus* amebocyte lysate assay. Fab of anti-CD137L mAb were prepared using immobilized papain (Pierce, Rockford, IL) and were purified using immobilized Protein L (Pierce). The purity of Fab was verified by SDS-PAGE analysis. mAbs against the following Ags were used for immunofluorescence analysis: CD3 (145-2C11, hamster IgG), CD4 (RM4-5, rat IgG2a), CD8 (53-6.7, rat IgG2a), CD45.1/Ly5.1 (A20, mouse IgG2a), CD45R/B220 (RA3-6B2, rat IgG2a), H-2K^d (SF1-1.1, mouse IgG2a), H-2K^b (AF6-88.5, mouse IgG2a), IFN-γ (XMG1.2, rat IgG1), and IL-4 (BVD4-1D11, rat IgG2b). All FITC-, PE-, and PerCP-conjugated mAbs were obtained from PharMingen (San Diego, CA). Multicolor staining for intracellular cytokine and cell surface Ags was performed as previously described (32). Flow cytometry and data analyses were performed using a FACSort, FACScalibur, and CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA). Hybridomas producing mAbs against I-A^{b,d,q} (M5/114), CD24 (HSA, J11d), CD45R/B220 (RA3-6B2), CD4 (RL172.4), and CD8 (3.155) were obtained from the American Tissue Culture Collection (Manassas, VA) and were used as culture supernatants.

Induction of acute and chronic GVHD and mAb treatment

For induction of aGVHD by BMT, recipient CBF1 mice were lethally irradiated (10.0 Gy), and BM cells (2.5 × 10^7) from B6 mice and splenocytes (2.5 × 10^7) from either B6 or B6/Ly5.1 mice were injected i.v. as described previously (26). A control group of mice received BM cells alone. The recipient mice received BM, and splenocytes (referred as BMS mice) were treated with control rat Ig (Sigma, St. Louis, MO), anti-CD137L mAb, or anti-CD80 and CD86 (CD80/86) mAbs. Two hundred micrograms of each mAb was administered i.p. on days 0, 1, and 2 and every other day until day 21 post-BMT for 12 times.

For induction of cGVHD, unirradiated BDF1 recipients received 1 × 10^8 splenocytes by i.v. injection. Two hundred micrograms of control rat Ig or anti-CD137L mAb was administrated i.p. three times a week for 4 wk. In some experiments DBA/2 splenocytes were treated with anti-I-A, anti-CD24, and anti-CD8 mAbs and rabbit compliment to deplete APC and CD8$^+$ T cells. As assessed by flow cytometry, this procedure resulted in <1% contaminating CD8$^+$ T cells and >85% purity of CD4$^+$ T cells. BDF1 recipients received 3 × 10^7 CD8-depleted T cells and were treated with mAb as described above.

Measurement of anti-host cytotoxicity

Individual splenocytes from GVHD mice were treated with anti-CD24, anti-I-A, and anti-CD4 mAbs and rabbit complement. Then, CD8-enriched T cell fractions were obtained and used as effector cells. Cytotoxicity against A20 (H-2^d) or EL-4 (H-2^b) was measured by a standard 6-h ^{51}Cr release assay.

Measurements of serum IgE and autoantibody

Mice were bled by retro-orbital venipuncture, and serum samples were individually aliquoted and stored at −80°C. Quantitations of total IgE and anti-dsDNA Ab were performed by ELISA as described previously (29, 33).

Measurement of IL-4 production in MLR

Splenocytes from BALB/c mice were treated with anti-CD24, anti-I-A, anti-CD45R/B220, and either anti-CD4 or anti-CD8 mAbs and rabbit complement. The purities of CD4$^+$ and CD8$^+$ T cell fractions was confirmed

to be >95% CD4$^+$ T cells and >90% CD8$^+$ T cells by flow cytometry. For MLR, CD4$^+$ T cells (1 × 10^6/well) were cocultured with mitomycin C-treated splenocytes (1 × 10^6/well) from B6 mice in 24-well plates in the presence or the absence of anti-CD137L (10 μg/ml) or anti-IFN-γ (R4-6A2, rat IgG1, 5 μg/ml). To determine the effect of CD8$^+$ T cells, CD8$^+$ T cells (0.5 × 10^6/well) were further added to the wells. On day 7, IL-2 (PharMingen; 100 U/ml) was added, and cells were cultured for an additional 2 days. Cells were harvested, rested in the medium alone for 8 h, and then restimulated in the anti-CD3 (2C11, 10 μg/ml) mAb-coated plates. After 48 h supernatants were collected for assessment of IL-4 production. ELISA for IL-4 was performed according to the protocols recommended by the manufacturer (*e*Bioscience, San Diego, CA).

Statistical analyses

Significant differences between experimental groups were analyzed by the Mann-Whitney test.

Results

Blockade of CD137 pathway by mAb ameliorates aGVHD, whereas it exacerbates cGVHD

It has been reported that CD137 is induced on T cells in allogeneic responses (34) and the administration of agonistic anti-CD137 mAb enhanced allogeneic CTL responses in aGVHD mice (6). To investigate the direct role of natural ligand for CD137, we first examined the effect of anti-CD137L mAb treatment on the development of both acute and chronic GVHD using parent into F$_1$ models. For aGVHD, BM and splenocytes from donor B6 mice were transferred into lethally irradiated CBF1 mice and were treated with anti-CD137L mAb for 3 wk just after BMT. All mice that received BM and splenocytes from B6 mice (referred to as BMS mice) showed significant weight loss and died by day 70, while mice that received BM cell alone (referred to as BM mice) survived >100 days, with a gradual increase in body weight (Fig. 1). Consistent with our previous report (26), treatment with anti-CD80/86 mAbs almost completely ameliorated the lethality of aGVHD. Treatment with anti-CD137L mAb failed to prevent early death by aGVHD within 1 mo; however, the survivors maintained their body weights as well as the anti-CD80/86-treated mice, and the final survival rate was 43% on day 100. We failed to observe a clear amelioration of lymphocyte recovery in peripheral blood and of histology in spleen (data not shown). These results demonstrated that blockade of CD137L interaction partially ameliorated the lethality of aGVHD.

Chronic GVHD was induced by injecting parent DBA/2 splenocytes into unirradiated BDF1 mice. Serum IgE and IgG anti-
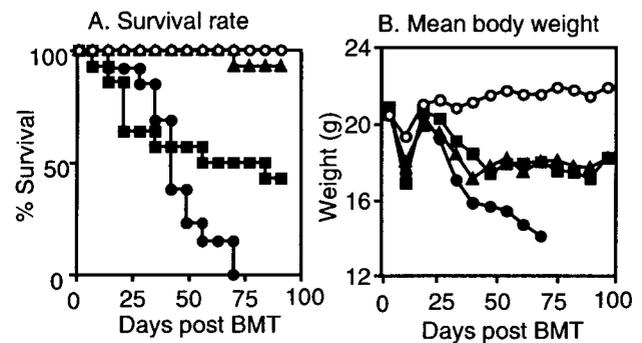
FIGURE 1. Amelioration of lethality by anti-CD137L mAb treatment in aGVHD. Lethal aGVHD was induced by transfer of 2.5 × 10^7 each of BM cells and splenocytes from B6 mice into lethally irradiated CBF1 mice. Control mice received BM cells alone (○). The mice received BM cells and splenocytes (BMS mice) that were treated with control rat Ig (●), anti-CD137L (♦), or anti-CD80/86 mAbs (▲) 12 times. Each group consists of 12–14 mice. Survival rates (*A*) and mean body weights (*B*) are shown. Data are representative of two independent experiments with similar results.

dsDNA Ab was undetectable in untransferred intact BDF1 mice (not shown). Transfer of DBA/2 splenocytes induced IgE and IgG anti-dsDNA autoantibody production at 2 wk, and this reached a maximum at 4 wk (Fig. 2, A and B). Surprisingly, the anti-CD137L mAb treatment enhanced the production of IgE and autoantibody, especially at the early time point. The evaluation of IgG subclasses of anti-dsDNA Ab revealed the predominant enhancement of IgG1 and IgG2b subclasses by anti-CD137L mAb treatment (Fig. 2C). Similar results were obtained by treatment with Fab of anti-CD137L mAb (Fig. 2D), suggesting that the elevated IgE production may not be due to direct activation of B cells by cross-linking of CD137L. These results suggest that blockade of the CD137 pathway by mAb treatment accelerated the development of cGVHD.

Blockade of the CD137 pathway efficiently inhibits donor CD8^{+} T cell expansion in both GVHD models

In both GVHD models maximum donor T cell expansion was observed by 1–2 wk (32, 35). Therefore, we examined the splenic phenotype at 2 wk after transfer. As shown in Fig. 3A, the total cell number in the spleen was clearly reduced in the control Ig-treated aGVHD mice, and anti-CD137L mAb treatment improved the reduction of splenocytes as well as in the mice treated with anti-CD80/86 mAbs. These results suggest that anti-CD137L mAb treatment improved the elimination of host cells by anti-host CTL. In the anti-CD137L mAb-treated mice, expansion of donor CD8^{+} T cells, but not CD4^{+} T cells, was significantly inhibited. On the

contrary, the mice treated with anti-CD80/86 mAb did not show a reduction in donor CD8^{+} T cells.

In cGVHD mice, the number of total splenocytes increased about 2-fold, producing splenomegaly. These manifestations were further enhanced by anti-CD137L mAb treatment (Fig. 3B). The percentage of donor (H-2[b−]) cells in the control Ig-treated mice was $37.6 \pm 13.8\%$, and this was inhibited to $17.1 \pm 2.7\%$ by anti-CD137L treatment, suggesting the expansion of host B cells

FIGURE 3. Inhibition of donor CD8^{+} T cell expansion by anti-CD137L mAb treatment in both acute and chronic GVHD. A, Acute GVHD was induced by transfer of 2.5×10^7 each of BM from B6 mice and splenocytes from B6-Ly5.1 mice into lethally irradiated CBF1 mice. Recipient splenocytes at 14 days after transfer were stained with either FITC-anti-Ly5.1 or FITC-anti-H-2K[d], with either PE-anti-CD8 or PE-anti-CD4 and PerCP-anti-CD3 mAbs, or with appropriate fluorochrome-conjugated control Ig. Ly5.1^{+}CD3^{+}, Ly5.1^{+}4^{+}, and Ly5.1^{+}8^{+} cells were counted as donor, T, CD4^{+} T, and CD8^{+} T cells, respectively. The number of total splenocytes and the percentages of the indicated cells in BM (▨), control BMS (☐), anti-CD137L-treated BMS (■), and anti-CD80/86-treated BMS (▦) mice are shown. The percentages of H-2[d−] donor cells were 83.8 ± 1.0, 92.1 ± 1.5, and $90.6 \pm 0.6\%$ in control, anti-CD137L-treated, and anti-CD80/86 mAb-treated BMS mice, respectively. B, The numbers of total splenocytes in age-matched intact BDF1 (▨), control (☐), and anti-CD137L-treated (■) cGVHD mice at 14 days after transfer were counted. Splenocytes were stained with FITC-anti-H-2K[b], with either PE-anti-CD8 or PE-anti-CD4 and PerCP-anti-CD3 mAbs, or with appropriate fluorochrome-conjugated control Ig. H-2[b−]CD3^{+}, H-2[b−]CD4^{+}, and H-2[b−]CD8^{+} cells were counted as donor, T, CD4^{+} T, and CD8^{+} T cells, respectively. All data represent the mean $\pm$ SD from five mice. ∗, Statistically different ($p < 0.05$).

FIGURE 2. Enhancement of IgE and autoantibody production by anti-CD137L mAb treatment in cGVHD. Chronic GVHD was induced by the 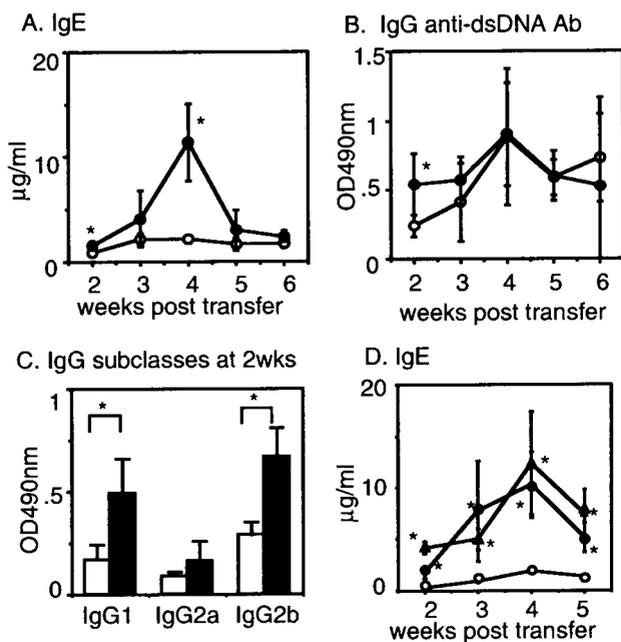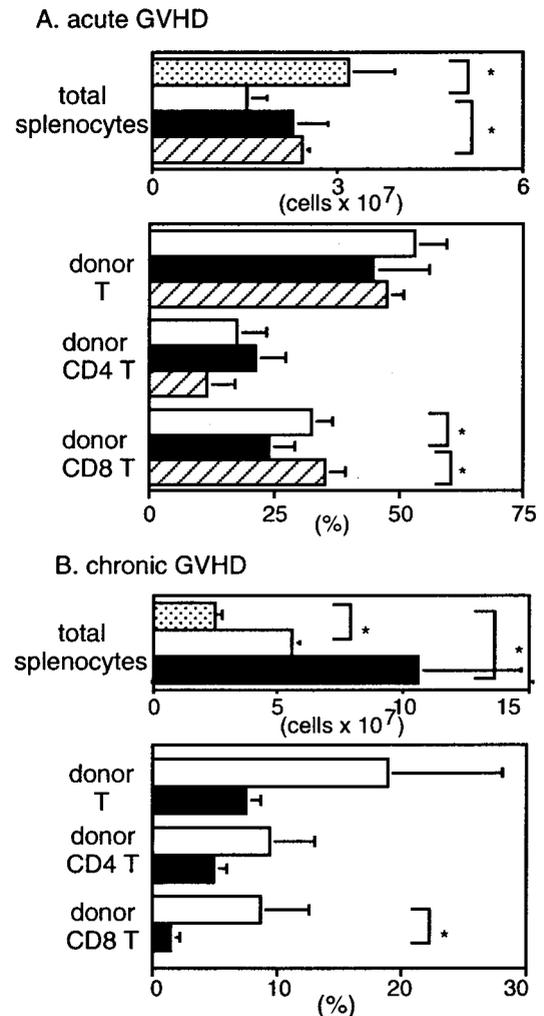transfer of DBA/2 splenocytes into unirradiated BDF1 mice. Two groups of four mice each were treated with either control Ig (open symbols) or anti-CD137L mAb (closed symbols). Two hundred micrograms of mAb or control Ig per mouse was injected i.p. 12 times. Total serum IgE (A) and total IgG of anti-dsDNA Ab (B) were determined at the indicated time points. C, IgG subclasses of anti-dsDNA Ab at 2 wk post-transfer are shown. D, Three groups of five mice were treated with either control Ig (○), anti-CD137L mAb (●), or Fab of anti-CD137L mAb (▲) as described above. Each value represents the mean $\pm$ SD, and the data are representative of two similar experiments. ∗, Statistically different from the control group ($p < 0.05$).

by anti-CD137L mAb treatment. Treatment with anti-CD137L mAb reduced donor T cell expansion; this was especially obvious in the CD8$^+$ T subset. These results suggest that anti-CD137L mAb treatment preferentially inhibits expansion of CD8 T cells, and this may result in preserved host cells and the activation of host B cells.

Functional ability by CD8$^+$ T cells is clearly inhibited by anti-CD137L mAb treatment

We next examined cytokine expression on donor T cells. In aGVHD mice anti-CD80/86 mAb treatment significantly inhibited IFN-γ expression by both CD4 and CD8 T cells, while anti-CD137L mAb treatment had a superior inhibitory effect in the CD8$^+$ subset (Fig. 4A). In cGVHD mice, although IFN-γ expression by CD8$^+$ T cells was efficiently inhibited as well as in aGVHD mice, both IFN-γ and IL-4 expression by CD4$^+$ T cells was enhanced by anti-CD137L treatment (Fig. 4B). To assess the cytolytic ability of CD8 T cells, we examined cytotoxicity against host alloantigen using a CD8$^+$-enriched fraction of recipient splenocytes at 14 days. As well as IFN-γ expression on CD8$^+$ T cells, cytotoxicity against host alloantigen was efficiently inhibited by anti-CD137L treatment in the aGVHD model, whereas the anti-CD80/86 mAb treatment did not have a significant effect (Fig. 5A). Consistent with previous reports (21, 35, 36), an obvious cytotox-
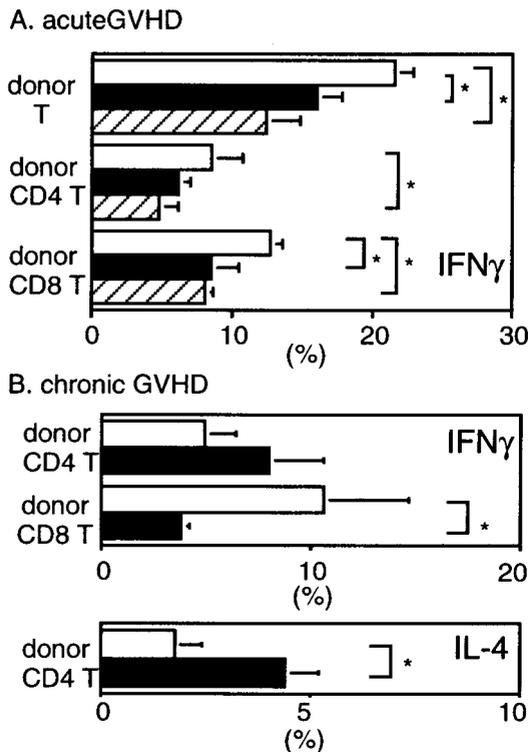

FIGURE 5. Inhibition of CD8$^+$ T cell-dependent cytotoxicity against host alloantigen by anti-CD137L mAb treatment in aGVHD. Individual splenocytes from control Ig (○, □), anti-CD137L mAb (●, ■), or anti-CD80/86 mAb (▲)-treated GVHD mice at 14 days were obtained. The CD8$^+$ T cell-enriched fraction was used as effector cells. The mean percentages of CD8$^+$ in the control Ig-, anti-CD137L-, and anti-CD80/86 mAb-treated mice are 65.4, 67.5, and 66.8% for aGVHD mice (A) and 91.4 and 89.9% for cGVHD mice (B), respectively. Cytotoxicity against A20 (H-2^d) and EL-4 (H-2^b) at the indicated E:T cell ratio was measured by a 6-h ^{51}Cr release assay. Data are representative of two experiments. *, Statistically different ($p < 0.05$).

icity against host alloantigen was not observed in the DBA/2-cGVHD mice, and cytotoxicity was not affected by anti-CD137L treatment (Fig. 5B).

CD8$^+$ T cells are involved in enhanced Ab and IL-4 production by blockade of CD137

To determine the contribution of donor CD8$^+$ T cells in enhancement of Ig production by anti-CD137L treatment in cGVHD mice, we examined the effect of anti-CD137L mAb treatment in a DBA-GVH model induced by CD8$^+$ T cell-depleted donor T cells. As shown in Fig. 6, A and B, the enhancement of IgE and autoantibody production by anti-CD137L treatment became mild without donor CD8$^+$ T cells. These results suggest a partial involvement of donor CD8$^+$ T cells in the acceleration of cGVHD by anti-CD137L treatment.

To further confirm the requirement for CD8$^+$ T cells in enhanced IL-4 production by the anti-CD137L mAb treatment, we examined the effects of addition of anti-CD137L mAb and the involvement of CD8$^+$ T cells and IFN-γ on IL-4 production in primary CD4$^+$ T cell MLR. As shown in Fig. 6C, CD4$^+$ T cells stimulated by allogeneic splenocytes produced moderate IL-4, and this was minimally induced by the addition of anti-CD137L mAb during primary responses. Interestingly, the addition of neutralizing anti-IFN-γ mAb dramatically enhanced IL-4 production. When CD8$^+$ T cells were added to similar MLR cultures, IL-4 production was clearly reduced. The addition of anti-CD137L mAb or anti-IFN-γ mAb partially rescued the reduced IL-4 production at similar levels. These results demonstrated that IFN-γ may play a key regulatory role in the generation of IL-4-producing CD4$^+$ T cells in a primary MLR, and the blockade of CD137L may preferentially inhibit CD8$^+$ T cell activation, resulting in reduced production of IFN-γ.

Discussion

We demonstrate that administration of anti-CD137 mAb ameliorates the lethality of aGVHD, whereas similar treatment exacerbates the manifestation of cGVHD. In both GVHD models anti-CD137L mAb treatment enhanced total cell number in the recipient spleen and efficiently inhibited donor CD8$^+$ T cell expansion and functional activation. In CD4$^+$ T cells, blockade of the CD137 pathway did not

FIGURE 4. Reduction of IFN-γ-expressing donor CD8$^+$ T cells by anti-CD137L mAb treatment. Individual splenocytes at 14 days after transfer in acute (A) and chronic (B) GVHD mice were assessed by immunofluorescent staining of intracellular cytokines by flow cytometry. Splenocytes were stained with either FITC-Ly5.1, FITC-anti-H-2K^b mAb, PerCP-anti-CD3, PerCP-anti-CD4, PerCP-anti-CD8 mAb, and PE-anti-IFN-γ or anti-IL-4 mAb or with appropriated fluorochrome-conjugated control Ig. Samples were analyzed by flow cytometry. An electronic gate was set on either Ly5.1$^+$ (A) or H-2^{b-} (B) donor splenocyte-derived cells, and the percentages of CD3$^+$IFN-γ^+, CD4$^+$IFN-γ^+, CD8$^+$IFN-γ^+, and CD4$^+$IL-4$^+$ cells in control Ig (□), anti-CD137L mAb (■), and anti-CD80/86 mAb (▨)-treated GVHD mice were analyzed. All data represent the mean ± SD from five mice. *, Statistically different ($p < 0.05$).
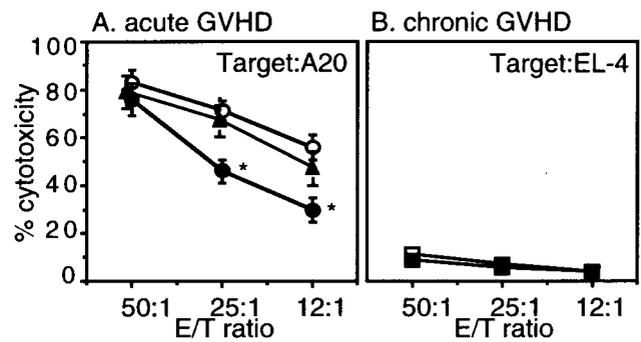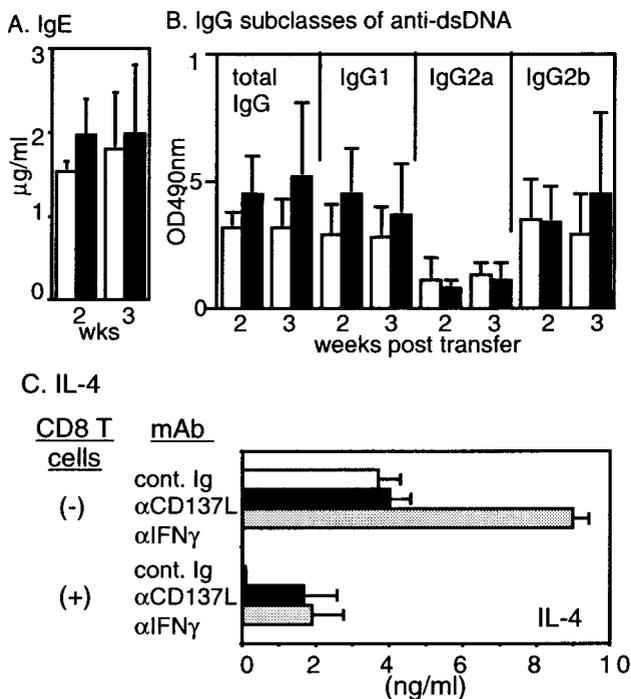
FIGURE 6. CD8$^+$ T cells are involved in the enhancing effects on production of IgE, autoantibody, and IL-4 by CD137 blockade. *A* and *B*, Chronic GVHD was induced by the transfer of CD8-depleted T cells (3 × 10^7) from DBA/2 mice into unirradiated BDF1 mice. Two groups of six mice each were treated with either control Ig (□) or anti-CD137L mAb (■) three times a week. Total serum IgE (*A*) and total IgG and IgG subclasses of anti-dsDNA (*B*) were determined. Each value represents the mean ± SD from six mice. *C*, IL-4 production by primary CD4$^+$ T cell MLR cultures in the presence or the absence of CD8$^+$ T cells was determined. Control Ig, anti-CD137L mAb, or anti-IFN-γ mAb was added to the initial cultures.

significantly affect CD4$^+$ T cell expansion in either model. Although controversial, in a Th2 cytokine-driven cGVHD, the same treatment enhanced the number of CD4$^+$ T cells expressing IL-4. Our results indicate the distinct contribution of the CD137 costimulatory pathway between CD4$^+$ and CD8$^+$ T cells and show the contrasting outcomes after blockade of CD137 in the development of both types of GVHD.

In our lethal aGVHD model, blockade of CD137L ameliorated the lethality, but it was not as efficient as blockade of CD80/86. Blockade of CD137 failed to prevent early death within 1 mo post-BMT. However, the survivors maintained their body weights comparable to the anti-CD80/86 mAb-treated group, and late death was not observed. These results suggest that the inhibitory effect of CD137 blockade is not sufficient in an initial GVH response, but is enough in the late responses of GVH reactions. The other characteristic result of CD137 blockade is less of an inhibitory effect on CD4$^+$ T cell expansion and activation despite the efficient suppression of CD8$^+$ T cell activation. CD137 is minimally expressed on resting T cells and is inducible on both activated CD4$^+$ and CD8$^+$ T cells. However, Tan et al. (34) reported a preferentially high and rapid induction of CD8$^+$ T cells after allogeneic stimulation and a requirement for multiple cell divisions for the induction of CD137 in both CD4$^+$ and CD8$^+$ T cells. Stimulation through CD137 by an agonistic anti-CD137 mAb dramatically augmented proliferation and IFN-γ production by CD8$^+$ T cells, but CD137 signals had a weak effect on proliferation of CD4$^+$ T cells (34). In contrast a recent report demonstrated that anti-CD137 mAb increased aGVHD induced by CD8$^+$ or CD4$^+$ T cells infused into sublethally irradiated MHC class I- or MHC class II-only disparate recipients (37). However, in their results prolifera-

tive responses and Th1 and Th2 cytokine production by CD4$^+$ T cells were not affected by the treatment. Therefore, we favor the preferential involvement of CD137 in CD8$^+$ T cell-mediated allogeneic responses. A prior report demonstrated that both acute and chronic GVHD share a common event, the recognition of allogeneic MHC class II molecules by donor CD4$^+$ T cells, which results in increased IL-2 production and proliferation during the first 2 days after parental cell transfer (38). However, the induction of CD137 on CD4$^+$ T cells is not enough at this early period; therefore, the blockade of CD137 by anti-CD137L mAb may be invalid at such a critical period for inhibition of initial CD4$^+$ T cell activation. This may result in partial amelioration by blockade of CD137 and a lesser effect on CD4$^+$ T cell inactivation in aGVHD mice.

In cGVHD our results showed that anti-CD137L mAb treatment induced obvious splenomegaly, early production of autoantibody and IgE, and increased numbers of IL-4-producing CD4$^+$ T cells, which are characteristic features of cGVHD. However, despite these observations donor T cell expansion was consistently inhibited by anti-CD137L mAb treatment, especially within the CD8 subset. Prior reports demonstrated the importance of the regulatory role of donor CD8$^+$ T cells in the development of cGVHD (35, 36, 39). We have also demonstrated a critical role of CD8$^+$ T cell expansion in the early response for regulation of the consequent Th2-mediated humoral immune responses in BALB/c GVH reactions (32). Our results suggest that blockade of CD137 preferentially inhibits CD8$^+$ T cell expansion and activation. This may reduce IFN-γ production, which regulates Th2 cell differentiation, and may result in the augmentation of Th2 cell differentiation and activation. This was further confirmed by the in vitro MLR experiments. By the addition of CD8$^+$ T cells, IL-4 production from primary CD4$^+$ T cell MLR cultures was clearly reduced, and this was enhanced by CD137L blockade as well as neutralization of IFN-γ. In the absence of CD8$^+$ T cells, IL-4 production was not affected by CD137 blockade, but neutralization of IFN-γ efficiently enhanced IL-4 production by CD4$^+$ T cells. This suggests that CD137 blockade may inhibit IFN-γ produced by CD8$^+$ T cells, but not by CD4$^+$ T and other cells. Consistently, depletion of donor CD8$^+$ T cells diminished the effect of anti-CD137L mAb treatment in the in vivo model of cGVHD, In the absence of donor CD8$^+$ T cells, the mice treated with anti-CD137L mAb still exhibited higher autoantibody and IgE production. These results suggest the involvement of other effector cells in this mechanism. NK and NKT cells may be possible candidates, since the expression and functional involvement of CD137 by NK and NKT cells have been shown (40, 41). Further studies will be required to clarify this issue. The prolonged administration with anti-CD137L mAb for 6 wk did not clearly improve the manifestation of cGVHD (not shown), suggesting the failure of blockade of already activated Th2 cells by anti-CD137L mAb treatment. Similarly, invalid effects have been shown in Th1- and Th2-mediated experimental leishmaniasis. The administration of anti-CD137L mAb was of no effect on either Th2 cell-mediated susceptibility in BALB/c mice or Th1 cell-mediated resistance in B6 mice (31). On the one hand, Mittler et al. (42) demonstrated that an initial treatment with an agonistic anti-4-1BB mAb inhibited T cell-dependent humoral immune responses independent of CD8$^+$ T cells, whereas the same treatment enhanced CD8$^+$ T cell-mediated aGVHD (6). Although we cannot simply reverse the results between the agonistic anti-CD137 mAb and the blocking anti-CD137L mAb, such a CD137-mediated inhibition in humoral immunity may overlap with the effect of CD137 blockade in CD8$^+$ T cells.

In this study we clearly demonstrated the effective inactivation of CD8$^+$ T cells in both acute and chronic GVHD and a lesser

effect on inactivation of $CD4^+$ T cells by blockade of CD137 costimulation. Our present results have confirmed the preferential function of CD137-mediated costimulation in $CD8^+$ T cell in acute and chronic GVHD models using an antagonistic anti-CD137L mAb. Among the multiple costimulatory molecules, CD137 may be a unique and powerful costimulator for $CD8^+$ T cells, but not for $CD4^+$ T cells. Intervention in the CD137 co-stimulatory pathway may be effective for some selected diseases in which $CD8^+$ T cells have the major effect or pathogenic cells. Otherwise, a combined approach will be required for intervention in residual functional $CD4^+$ T cells.

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