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Induction of Lethal Graft-versus-Host Disease by Anti-CD137 Monoclonal Antibody in Mice Prone to Chronic Graft-versus-Host Disease

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Chronic graft-versus-host disease (cGVHD) is an increasingly frequent complication of allogeneic stem cell transplantation. We previously showed that anti-CD137 monoclonal antibody (mAb) can cure advanced cGVHD by inducing activation-induced cell death of donor T cells. In this study, we examined whether administration of anti-CD137 mAb can prevent the development of cGVHD after bone marrow transplantation (BMT) in mice conditioned with total body irradiation (TBI). We used the B10.D2 → Balb/c (H-2^d) minor histocompatibility antigen-mismatched model, which reflects clinical and pathological symptoms of human cGVHD. A single injection of anti-CD137 mAb was administered immediately after BMT. In contrast to the results obtained from the curing model of cGVHD, anti-CD137 given simultaneously with BMT resulted in lethal GVHD. Histopathologic evaluation revealed inflammation and damage of target organs from acute GVHD (aGVHD) in anti-CD137-treated mice. Anti-CD137–induced lethal aGVHD required host cells, as well as irradiation and mature donor T cells. Apparently, anti-CD137 mAb rapidly induced activation of donor T cells and sustained their activation status under the inflammatory condition triggered by irradiation. When given on day 12 after irradiation and BMT, anti-CD137 mAb could still exacerbate GVHD, but when given on day 30, it could not. Our data demonstrate that anti-CD137 mAb can amplify inflammation induced by host preconditioning, subsequently resulting in lethal aGVHD; thus, alleviating irradiation-induced toxicity is critical to allow the use of anti-CD137 mAb as GVHD prophylaxis.

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INTRODUCTION

CD137 is a member of the tumor necrosis factor (TNF) receptor superfamily that functions mainly as a strong co-stimulatory molecule for $CD8^+$ T cells [1,2]. Since Mittler et al. [3] demonstrated that in vivo ligation of CD137 abrogates T cell–dependent antibody responses, an explosion of studies have demonstrated the preventive and/or therapeutic effect of agonistic anti-CD137 monoclonal antibody (mAb) on various clinical settings of autoimmune/inflammatory diseases [4-15]. But despite the impressive preventive/therapeutic effect of anti-CD137 mAb on spontaneous or experimentally induced diseases, the underlying mechanisms remain inconclusively defined. Increasing evidence suggests that anti-CD137 mAb strongly promotes the activation-induced cell death (AICD) of antigen-specific T cells (including autoreactive and pathogenic T cells) [4,9,10,12,15,16], which may or may not result in $CD4^+$ T cell tolerance [3,9,13]. Anti-CD137 mAb also can induce the deletion of autoreactive B cells by augmenting the production of interferon (IFN)-γ by $CD8^+$ T cells [5,7,9]. The therapeutic effect of anti-CD137 mAb remains intact or decreases mildly in the absence of $CD8^+$ T cells in various disease models, however [9,13-16].

We previously showed that anti-CD137 mAb inhibits the induction of chronic graft-versus-host disease (cGVHD) in the DBA/2 → unirradiated (C57BL/6 × DBA/2)F1 (BDF1) mice [9]. Surprisingly, anti-CD137 mAb has been shown to be highly effective in reversing already established cGVHD in the B10.D2 → Balb/c (H-2^d) minor histocompatibility antigen-mismatched model of cGVHD [15]. This

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disease has characteristics resembling those of human cGVHD, including relatively late onset, skin fibroses, ulcerations, and alopecia, with increased collagen deposition [17-20]. Treatment of anti-CD137 mAb between day 30 and day 60 after disease induction has markedly reduced the symptoms of cGVHD. The Fas-mediated AICD of donor $CD4^+$ T cells is required for the therapeutic effect of anti-CD137 mAb on cGVHD. These findings suggest co-stimulation as a possible therapeutic intervention in cGVHD. Because anti-CD137 mAb strongly induces the AICD of alloreactive donor T cells in parent→unirradiated F1 hybrid mice [9], these findings also suggest that anti-CD137 mAb may be used as GVHD prophylaxis.

In this study, we used the B10.D2→Balb/c cGVHD model to assess the preventive effect of anti-CD137 mAb on cGVHD. We found that during the induction and initial phase of cGVHD, anti-CD137 mAb induced lethal aGVHD, which was associated with increased levels of TNF-α and IFN-γ in the large intestine. Treatment with anti-CD137 mAb rapidly activated donor $CD4^+$ T cells and activated weakly alloreactive donor $CD8^+$ T cells. Overall, anti-CD137 mAb intensified the inflammation triggered by total body irradiation (TBI) and drove the disease toward systemic lethal GVHD in mice that otherwise were prone to cGVHD. Our results suggest that an inflammatory environment after TBI inhibits the immunosuppression induced by anti-CD137 mAb.

MATERIALS AND METHODS

Mice and Antibodies

Male B10.D2 ($H\text{-}2^d$) donor mice were purchased from Japan Shizoka Institute for Laboratory Animals, Hamamatsu, Japan. Balb/c recipient mice were purchased from Orient, Seoul, Korea. All mice were age 6 to 8 weeks and were maintained in pathogen-free conditions. Anti-CD137 (3H3) mAb [1] was purified from ascites. Control rat IgG (Ig) was purchased from Sigma-Aldrich, St. Louis, MO. The study design was approved by the institutional Animal Care Committee.

Bone Marrow T Cell Depletion

Bone marrow (BM) cells were collected by flushing femurs and tibias from B10.D2 donor mice into MACS buffer (1 × phosphate-buffered saline [PBS], 5 mM EDTA, and 3% calf serum). After erythrocyte lysis in hemolysis buffer (144 mM NH4Cl and 17 mM Tris-HCl [pH 7.2]), BM cells were incubated with biotinylated anti-CD3 mAb for 20 minutes on ice, washed once, then incubated with streptavidin-conjugated microbeads (Miltenyi Biotech, Auburn, CA) for 20 minutes at 4°C. Cells were depleted of $CD3^+$ cells using magnetic-activated cell separation (MACS; Miltenyi Biotech). The remaining $CD3^+$ cells routinely composed < 1% of the BM cells. Cells were resuspended in PBS before transplantation.

$CD4^+$ and $CD8^+$ T Cell Purification

Single-cell suspensions in PBS were prepared from the spleens and lymph nodes of normal B10.D2 parental donors, filtered through a sterile mesh (Falcon; BD Biosciences, San Diego, CA) and washed. After the erythrocytes were lysed in hemolysis buffer, the remaining cells were resuspended in MACS buffer. $CD4^+$ or $CD8^+$ T cells were purified using anti-CD4– or anti-CD8–conjugated magnetic beads (Miltenyi Biotech). Positively selected cells routinely contained > 90% $CD4^+$ or $CD8^+$ T cells.

Bone Marrow Transplantation

Recipient Balb/c mice received 750 cGy from a cesium irradiator and were reconstituted with 5×10^6 of T cell-depleted BM with or without 1×10^7 of purified $CD4^+$ T cells from B10.D2 donors. In some experiments, cGVHD was induced by transferring 5×10^6 of donor T cell–depleted BM and 6×10^6 of purified $CD8^+$ T cells or 6×10^6 total spleen/lymph node cells. A single i.p. injection of anti-CD137 mAb or control Ig (200 μg per mouse) was given at various time points after BM transplantation (BMT).

cGVHD Clinical Scoring System

cGVHD was evaluated as described previously [21]. In brief, after BMT, the mice were weighed every 3 days and scored for skin manifestations of GVHD beginning on day 15. The following scoring system was used: 0, healthy appearance; 1, skin lesions with alopecia < 1 cm^2 in area; 2, skin lesions with alopecia 1 to 2 cm^2 in area; 3, skin lesions with alopecia > 2 cm^2 in area. In addition, the mice were assigned 0.3 point each for skin disease (lesions or scaling) on the ears, tails, and paws. The minimum score was 0, and the maximum score was 3.9. Incidence curves represent all mice that achieved a score of 0.6 or higher and mean clinical scores were calculated for all mice used for experiments per group.

Histology

Shaved skins from the interscapular region (approximately 2 cm^2), livers, and colons were fixed in 10% formalin, embedded in paraffin, sectioned, slide-mounted, and stained with H & E or Masson's trichrome.

Pathological Scoring of GVHD

Formalin-fixed livers and distal colons were embedded in paraffin, and 5-mm-thick sections were stained with H & E for histological examination. Slides