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Prevention of Acute Graft-versus-Host Disease in a Xenogeneic SCID Mouse Model by the Humanized Anti-CD74 Antagonistic Antibody Milatuzumab

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Prevention and treatment of graft-versus-host disease (GVHD) remains a major challenge, given that current T-cell depletion and mainstay immunosuppressive therapies compromise preexisting T-cell immunity, often leading to severe infections and disease relapse. Thus, there is a critical need for novel anti-GVHD agents that can spare protective T-cell memory. Here we show that milatuzumab (hLL1), a humanized anti-CD74 antagonist monoclonal antibody, can moderately reduce the numbers of CD74-expressing B cells and myeloid dendritic cells, but has no effect on the survival of T cells that are $CD74^-$. Consequently, milatuzumab inhibits allogeneic T-cell proliferation in mixed leukocyte reactions. In a human/mouse xenogeneic SCID mouse model in which GVHD is induced and mediated by engrafted human $CD4^+$ T cells and dendritic cells, milatuzumab effectively prevents the onset and manifestations of acute GVHD, suppresses serum levels of human IFN-γ and IL-5, eliminates the infiltration of human lymphocytes into GVHD target organs (ie, lung, liver, and spleen), and significantly promotes survival (90% versus 20% for controls; $P = .0012$). Importantly, exposure to milatuzumab does not affect the number of cytomegalovirus-specific, IFN-γ–producing human $CD8^+$ T cells in allogeneic mixed leukocyte reactions. These encouraging results warrant further exploration of milatuzumab as a possible new therapeutic agent for GVHD.

INTRODUCTION

Graft-versus-host disease (GVHD) is the major cause of mortality and morbidity in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a powerful and currently the sole curative therapy for many malignant and nonmalignant hematologic diseases [1]. Prevention and treatment of GVHD remains a major challenge [2], given that contemporary T-cell depletion and immunosuppressive therapies, although effective in controlling GVHD in certain patients, compromise preexisting T-cell immunity, leading to severe infections and disease relapse [3-6]. Thus, there is a critical need for novel anti-GVHD agents that spare protective T-cell memory.

Milatuzumab (hLL1) is a humanized IgG1κ mAb that reacts with human CD74 [7-9], the HLA class II–associated invariant chain [10]. Previous studies found that milatuzumab has potent cytotoxicity against CD74-expressing malignant B cells in vitro and in xenograft models [7,11], which has led to the ongoing clinical evaluation of milatuzumab in relapsed or refractory B-cell malignancies [12]. More recent preclinical studies [13] have demonstrated that milatuzumab is capable of modulating human B cell proliferation, migration, and adhesion molecule expression, suggesting the therapeutic potential of this mAb in autoimmune diseases.

As an HLA class II invariant chain molecule, CD74 is widely expressed in both hematopoietic and nonhematopoietic antigen-presenting cells (APCs), which include B cells, monocytes, macrophages, Langerhans cells, dendritic cells (DCs), and endothelial and certain epithelial cells [7,14]. Because both recipient and donor APCs, including nonhematopoietic APCs, play critical roles in the initiation of GVHD [15-24], we reasoned that milatuzumab might have therapeutic potential for GVHD by affecting recipient and/or donor APCs. The anti-GVHD potential of anti-CD74 mAb is also supported by evidence that macrophage migration inhibitory factor (MIF), the ligand of CD74 [25], is involved in the development of acute GVHD in a murine model of allogeneic stem cell transplantation [26].

In this study, we demonstrate that milatuzumab selectively reduces myeloid DCs (mDCs) and B cells, but not plasmacytoid DCs (pDCs), monocytes, or T cells, in human peripheral blood mononuclear cells (PBMCs). As a result, milatuzumab inhibits allogeneic mixed lymphocyte reactions (allo-MLRs); and in a human PBMC-transplanted SCID mouse model (hu-PBL-SCID) [27], milatuzumab effectively prevents acute GVHD, suppresses human cytokine storm, eliminates infiltration of human lymphocytes in GVHD target organs, and significantly improves survival. Unlike alemtuzumab, an anti-CD52 mAb currently used clinically for GVHD prevention, milatuzumab does not affect cytomegalovirus (CMV)-specific T-cell immunity in vitro, suggesting that it might be exploited as a novel agent for GVHD without compromising preexisting antiviral immunity.

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MATERIALS AND METHODS

Antibodies and Reagents

Milatuzumab (hLL1), labetuzumab (hMN-14; a humanized anti-CEACAM5 mAb) [28], epratuzumab (hLL2; a humanized anti-CD22 mAb) [29], IMMU-T3 (a humanized anti-CD3 mAb), and hLL1-Fab-A3B3 (a fusion protein of milatuzumab Fab fragment with CEACAM5 [CD66e] A3B3 domain

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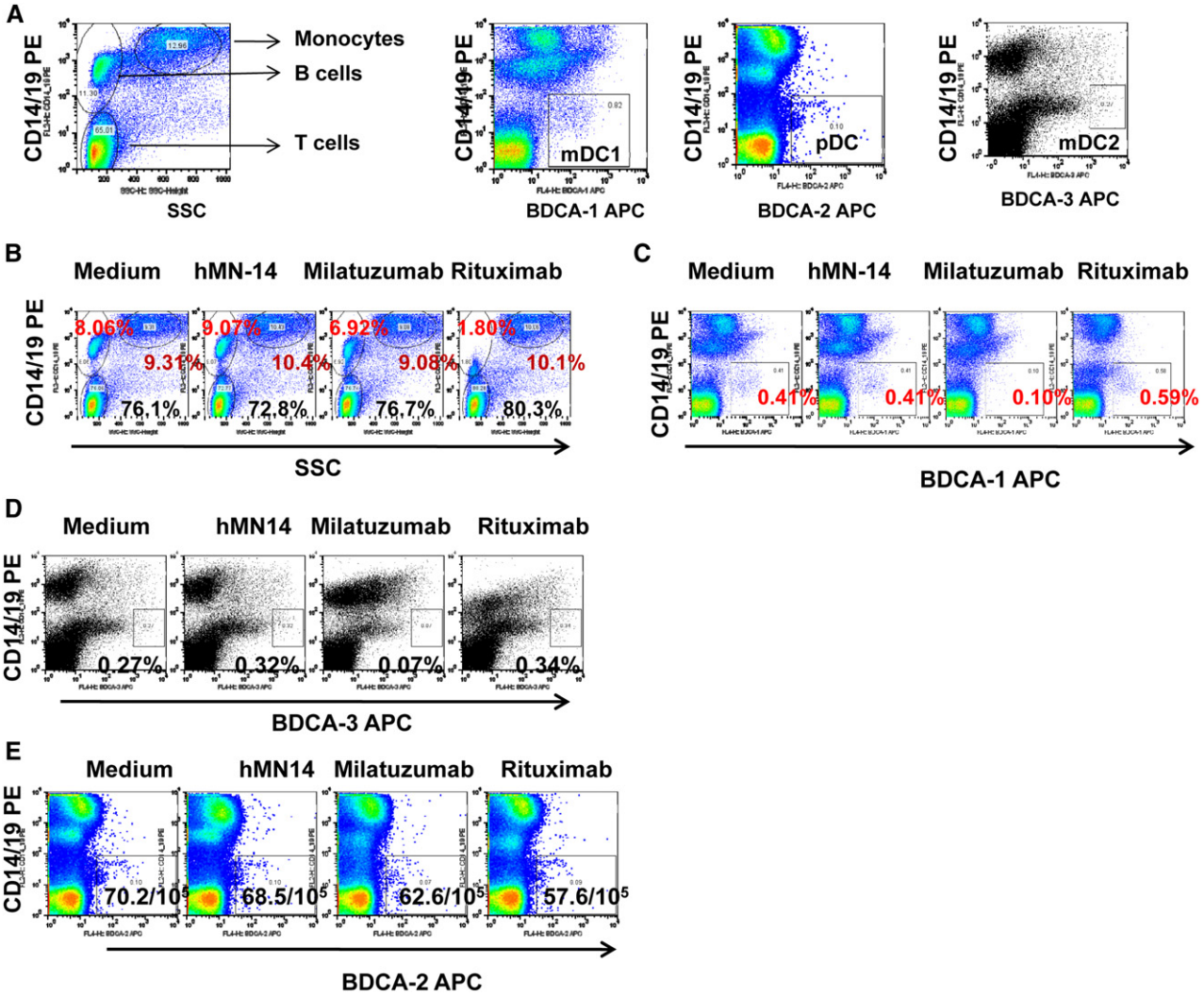

Figure 1. Milatuzumab selectively reduces myeloid DCs in human PBMCs. Human PBMCs were incubated with 5 μg/mL of milatuzumab, control mAbs, or medium only for 3 days. The effect of each treatment on APC subsets was evaluated by costaining the cells with PE-labeled anti-CD14 and anti-CD19 IgG, in combination with allophycocyanin-labeled anti–BDCA-1 IgG, for analysis of mDC1, or a mixture of FITC-labeled anti–BDCA-2 and allophycocyanin-labeled anti–BDCA-3 IgG for simultaneous analysis of mDC2s and pDCs, respectively. PBMCs were gated to exclude the debris and dead cells on the basis of their forward scatter and side scatter characteristics. (A) Subpopulations of PBMCs were gated as follows: monocytes, $CD14^{+}SSC^{medium}$; B cells, $CD19^{+}SSC^{low}$; T cells, $CD19^{-}CD14^{-}SSC^{low}$; mDC1, $CD14^{-}CD19^{-}BDCA\text{-}1^{+}$; mDC2, $CD14^{-}CD19^{-}BDCA\text{-}3^{++}$; pDCs, $CD14^{-}CD19^{-}BDCA\text{-}2^{+}$. (B-E) Representative flow cytometry data for B cells (B), monocytes (B), non-B lymphocytes (B), mDC1s (C), mDC2s (D), and pDCs (E) in PBMCs after mAb treatment. (F-H) Mean percentages of live mDC1s, mDC2s, and pDCs in PBMCs after mAb treatment (n = 7 donors). Error bars indicate SD. *P* values were determined by the paired *t* test. $^{**}P<.05$; $^{***}P<.01$ versus hMN-14 control.

[30]), were provided by Immunomedics (Morris Plains, NJ). Rituximab (anti-CD20) was purchased from IDEC Pharmaceuticals (San Diego, CA). Alemtuzumab (anti-CD52) was obtained from Genzyme (Ridgefield, NJ). The mAbs used for flow cytometry were obtained from BD Pharmingen (San Jose, CA): FITC-labeled anti-CD74 (M-B741), FITC-labeled mouse IgG1, PE-labeled anti-CD25, PerCp-labeled anti-CD8, and allophycocyanin-labeled anti-CD4 and CD3; or from Miltenyi Biotec (Auburn, CA): PE-labeled mAbs to CD19 (LT19) and CD14 (TÜK4) and allophycocyanin-labeled mAbs to BDCA-1 (AD5-8E7), BDCA-2 (AC144), and BDCA-3 (AD5-14H12). Human IgG was obtained from Jackson ImmunoResearch (West Grove, PA). HLA-A*0201 restricted CMV pp65 peptide (NLVPMVATV) and HIV gag peptide (SL9, SLYNTVATL) were obtained from Anaspec (Fremont, CA).

Assessment of APC Subsets in PBMCs

Buffy coats from expired whole blood were obtained from healthy donors at the Blood Center of New Jersey (East Orange, NJ), after approval by the New England Institutional Review Board. PBMCs were isolated from buffy coats by standard density-gradient centrifugation over Ficoll-Paque. The isolated PBMCs were treated with milatuzumab or other mAbs at 37°C in 5% CO_2 for 3 days. After incubation, the cells were stained with PE-labeled anti-CD14 and anti-CD19 in combination with allophycocyanin-labeled anti-BDCA-1. After washing, the cells were analyzed by flow cytometry using the gating strategy described below. Live PBMCs were gated based on forward scatter and side scatter signals. Within live PBMCs, type 1 mDCs (mDC1s) were identified as $CD14^{-}19^{-}BDCA\text{-}1^{+}$ cell populations [31], and the lymphocyte population was analyzed for B cells ($CD19^{+}SSC^{low}$), non-B lymphocytes (primarily T cells) ($CD19^{-}14^{-}SSC^{low}$), and monocytes ($CD14^{+}SSC^{medium}$). To measure the frequencies of pDCs and type 2 mDCs (mDC2s), PBMCs were stained with PE-labeled anti-CD14 and anti-CD19 IgG, in combination with FITC-labeled anti–BDCA-2 and allophycocyanin-labeled anti–BDCA-3 IgG. Within the live PBMCs, mDC2s were identified as the $CD14^{-}19^{-}BDCA\text{-}3^{+}$ cell population, whereas pDCs were identified as the $CD14^{-}19^{-}BDCA\text{-}2^{+}$ cell population [31]. Flow cytometry was performed using a FACSCalibur (BD Biosciences, San Jose, CA), and data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Assessment of T-Cell Apoptosis and Survival

Human PBMCs were treated with hMN-14, milatuzumab, or alemtuzumab at different concentrations for 18 hours in the presence of phytohemagglutinin (final concentration, 2.5 μg/mL) and IL-2 (final concentration,