

D-mannose ameliorates autoimmune phenotypes in mouse models of lupus

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Abstract

Background

Systemic lupus erythematosus is a disorder of immune regulation characterized by overproduction of autoantibodies. D-mannose is a C-2 epimer of glucose that exhibits immunoregulatory effects in models of autoimmune diseases, such as type 1 diabetes, induced rheumatoid arthritis, and airway inflammation. This study was conducted to evaluate the efficacy of D-mannose treatment in mouse models of lupus.

Methods

The effect of D-Mannose was evaluated by flow cytometry on the *in vitro* activation of C57BL/6 (B6) murine bone marrow derived dendritic cells and their ability to induce antigen specific CD4⁺ T cell proliferation and activation. The effect of D-mannose administration *in vivo* on the frequency of Foxp3⁺ regulatory T cells in B6 mice was assessed by flow cytometry. D-mannose was administered to two models of lupus: the chronic graft-versus-host disease (cGVHD) induced model and the B6.lpr spontaneous model. Autoantibody production was measured by ELISA and immune activation by flow cytometry. Results were compared by two-tailed statistics: unpaired or paired t tests, or Mann-Whitney U tests depending on whether the data was normally distributed.

Results

D-mannose inhibited the maturation of bone marrow dendritic cells and their induction of antigen-specific T cell proliferation and activation *in vitro*. *In vivo*, D-mannose increased the frequency of Foxp3⁺ regulatory T cells in unmanipulated control mice. In the cGVHD model of induced lupus, D-mannose treatment decreased autoantibody production, with a concomitant reduction of the frequency of effector memory and follicular helper T cells as well as germinal center B cells and plasma cells. These results were partially validated in the B6.lpr model of spontaneous lupus.

Conclusion

Overall, our results suggest that D-mannose ameliorates autoimmune activation in models of lupus, at least partially due to its expansion of Treg cells, the induction of immature conventional dendritic cells and the downregulation of effector T cells activation. D-Mannose showed however a weaker immunomodulatory effect in lupus than in other autoimmune diseases.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with a diverse clinical presentation. Tissue damage is mediated by pathogenic autoantibodies, however, abnormalities in cell development and function are not limited to B cells. Indeed, most types of immune cells are involved in SLE pathogenesis [1]. Among them, autoreactive CD4⁺ T cells, which have altered signaling and functions, play an essential role in several disease processes [2]. Lupus CD4⁺ T cells also present a skewed metabolic program that is directly associated with these immune dysregulations [3]. Simultaneous administration of the mitochondrial metabolism inhibitor metformin and the glucose metabolism inhibitor 2-deoxy-D-glucose (2DG) ameliorated disease severity in several strains of lupus-prone mice, which strongly suggests that manipulating metabolism in lupus may offer novel therapeutic options [4].

D-mannose, a C-2 epimer of glucose, has a physiological blood concentration of less than 2% of that of glucose [5]. However, when imported into cells, D-mannose interferes with the metabolism of glucose [6]. In CD4⁺ T cells, D-mannose decreases glycolysis and promotes fatty acid oxidation (FAO), which is associated with regulatory T (Treg) cell suppressive phenotypes [7]. Indeed, D-mannose promoted the expansion in the frequency of Treg cells *in vitro* and *in vivo* [8]. Accordingly, administration of D-mannose in drinking-water suppressed the immunopathology of autoimmune diabetes and airway inflammation in mouse models [8]. However, it is unknown whether the administration of D-mannose could suppress immunopathology in models of lupus.

Lupus development is associated with changes in the dendritic cell (DC) compartment, including altered frequency and localization of DC subsets, overactivation of conventional (cDCs) and plasmacytoid (pDC) DCs, as well as functional defects [9]. The mannose receptor (MR, also known as CD206) is an endocytic receptor involved in antigen presentation. The expression of CD206 on DCs impaired CD8⁺ T-cell cytotoxicity by direct interaction with CD45, and up-regulation of CTLA on the T cells [10]. In murine collagen-induced arthritis, blocking CD206 attenuated disease by modulating the differentiation, maturation, and functions of DCs, which shifted CD4⁺ T cell polarization from a Th1 / Th17 to Th2 cell [11]. It remains unclear whether D-mannose treatment in drinking water could regulate DCs function in a similar fashion.

Here, we show that D-mannose enhanced the expression of CD206 and Fcγ receptor in bone marrow-derived dendritic cells (BMDCs), which are the characteristics of immature DCs, and decreased their ability to activate CD4⁺ T cells. *In vivo*, we assessed the effects of D-mannose treatment in the chronic graft-versus-host disease (cGVHD) model, a well characterized induced model of lupus [12] as well as in the spontaneous B6.lpr model. We showed that D-mannose alleviated CD4⁺ T cell activation, expanded the frequency of Treg cells, restrained DCs activation and ameliorated lupus phenotypes. These results suggest an immunoregulatory effect of D-mannose in lupus models, which may provide a therapeutic potential in SLE patients.

Material And Methods

Mice

C57BL/6J (B6) mice, B6(C)-H2-Ab1^{bm12}/KhEgJ (bm12), B6.CgTg (TcraTcrb) 425Cbn/J (OT-II), B6.SJL-*Ptprc^a Pepc^b*/BoyJ (B6.SJL) and B6.MRL-*Fas^{lpr}*/J (B6.lpr) mice were obtained from the Jackson Laboratory. All mice were bred and maintained at the University of Florida in specific pathogen-free conditions. Both males and females were used between 2 and 6 months of age. Gender and age were matched for each experiment. D-mannose (Sigma) was dissolved at 1.1 M in drinking water and administered *ad lib*.

cGVHD induction and analysis

Chronic graft-versus-host disease (cGVHD) was induced according to an established protocol [12]. Briefly, $7-9 \times 10^6$ CD4⁺ T cells isolated by negative selection (Miltenyi) from spleen and mesenteric lymph nodes (mLN) of bm12 mice were transferred intraperitoneally into B6 or B6.SJL mice. bm12 CD4⁺ T cells are sufficient to initiate cGVHD in B6 mice [13], which allowed us to focus on the effect of D-mannose on donor CD4⁺T cells instead of mixed cell populations from the whole spleen. Serum was collected weekly up to 3 weeks after induction, at which point mice were sacrificed to assess immunophenotypes.

Flow cytometry

Single-cell suspensions were prepared from spleens and mLN using standard procedures. After RBC lysis, cells were stained in staining buffer (2.5% FBS, 0.05% sodium azide in PBS). Fluorochrome-conjugated Abs purchased from BD Biosciences, eBioscience, and BioLegend with the following specificities were used: CD3e (145-2C11), CD4 (RM4-5 and GK1.5), CD8 α (53-6.7), CD11b (M1/70), CD11c (HL3), CD19 (6D5), CD25 (PCX61 and PC61.5), CD32b (AT130-2), CD40 (3/23), CD44 (IM7), CD45.2 (104), CD62L (MEL-14), CD64 (X54-5/7.1), CD69 (H1.2F3), CD80 (16-10A1), CD86 (GL1), CD95 (15A7), CD122 (TM-b1), CD132 (TUGm2), CD138 (281-2), CD206 (C068C2), B220 (RA3-6B2), Bcl-6 (K112-91), CXCR5 (2G8), Foxp3 (FJK-16S), GITR (DTA-1), GL-7 (GL-7), IgD (217-170), Ki-67 (16A8), PD-1 (RMP1-30), PDCA-1 (927), and MHC-II (M5/114.15.2). CD4⁺ follicular helper T (T_{FH}) cells were stained as previously described [4] in a three-step process using purified CXCR5 (2G8) followed by biotinylated anti-rat IgG (Jackson Immunoresearch) and PerCP5.5-labeled streptavidin. Dead cells were excluded with fixable viability dye (eFluor780; eBioscience). Data were collected on LSRFortessa (BD Biosciences) and analyzed with FlowJo (Tree Star) software. IFN γ , IL-10 and IL-17A production were analyzed in cells treated with the leukocyte activation cocktail (BD Biosciences) for 5 h and the Fixation/Permeabilization kit (eBioscience). To avoid batch effect, mean fluorescence intensity (MFI) was reported as fold DMFI, defined as the MFI value in each sample divided by the mean MFI value in all controls. Similarly, the percent expression of makers in BMDC cultures was reported as fold D% defined as the percentage in each sample divided by the mean percentage in control BDMCs treated with 10 mM glucose (G10).

Generation of bone marrow-derived DCs (BMDCs) and BMDC/T cell co-cultures

Bone marrow single cell suspensions from B6 mice were cultured in RPMI-1640 supplemented with 10% FBS and 20 ng/mL of GM-CSF (R&D systems) for 5 d. Half of the media was replenished on d3. On d5,

the medium was changed to complete glucose-free RPMI-1640 with 10% FBS, supplemented with 10 mM D-mannose (M10), 10 mM glucose (G10), or their combination (G10M10) for 24 h. In some experiments, LPS (0.1 µg/mL) was added to the medium for 24 h on d5. For OVA-specific proliferation assays, M10, G10 or G10M10 treated BMDCs (5×10^4) were pulsed with 30 µg/ml ovalbumin (OVA, Sigma) for 2 h in complete RPMI-1640. Then, these BMDCs were incubated with Cell Trace Violet (CTV, Invitrogen Life Technologies) labeled OT-II CD4⁺ T cells (5×10^5) in either G10 or G10M10 medium. After 96 h of culture, T cell proliferation was measured as the CTV dilution by flow cytometry.

Antibody measurements

Serum autoantibody levels were determined as previously described by ELISA [14]. Briefly, anti-dsDNA IgG was measured in 1:100 diluted sera in duplicates and in plates coated with 50 µg/mL dsDNA (Sigma). The secondary antibody was goat anti-mouse IgG conjugated to alkaline phosphatase (Southern Biotech).

Gene expression assays

RNA was isolated from M10, G10 or G10M10-treated BMDCs with the RNeasy Mini Kit (Qiagen). qRT-PCR was performed with ImProm II Reverse Transcriptase (Promega) and SYBR Green Dye (Bio-RAD) on the BioRad CFX connect system with primer sequences shown in Sup. Table 1. The PCR thermo-cycling protocol was 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C repeated for 42 cycles. Expression was calculated using the $2^{\Delta Cq}$ method with difference in Cq values normalized to the housekeeping gene *Ppia* against the genes of interest.

Statistical analysis

Differences between groups were evaluated by two-tailed statistics: unpaired or paired t tests, or Mann-Whitney U tests depending on whether the data was normally distributed. The results are expressed as means \pm S.E.M. The statistical analyses were performed with the Graphpad Prism 7.0 software. The level of statistical significance was set at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Results

D-mannose inhibited BMDCs activation and BMDC-induced antigen-specific T-cell responses *in vitro*

An *in vitro* study was carried out to evaluate the effect of D-mannose relative to glucose on the function of BMDCs. The viability of BMDCs greatly decreased after a 24 h culture in a glucose-free culture medium with 10 mM D-mannose alone (Fig. 1A). Since a glucose-free condition is not physiologically relevant, we compared 10 mM glucose (G10) to the combination of 10 mM glucose and D-mannose (G10M10) in culture media. The addition of D-mannose to glucose enhanced the expression of CD206 (Fig. 1B) whether or not the BMDCs were activated with LPS, without affecting cell viability (data not shown). The expression of CD32b (FcγRIIb) and CD64 (FcγRI), associated with inhibitory function and antigen uptake,

respectively [15], were also increased by the addition of D-mannose, even in the presence of LPS stimulation (Fig. 1C-D). Notably, this effect of D-mannose on BMDCs was observed without altering the expression of activation markers (CD40, CD80 and CD86, data not shown). Finally, the mRNA expression of pro-inflammatory cytokines, *IL-6*, *TNF- α* and type 1 IFN (detected through the expression of the interferon stimulated gene *Mx1*), decreased after D-mannose treatment in LPS-activated BMDCs (Fig. 1E-G).

In light of the critical role of DCs in priming T cells, we next investigated the effect of D-mannose on the antigen-presenting function of OVA-loaded BMDCs co-cultured with OVA-specific OT-II CD4⁺ T cells (Fig. 1H). The proliferation of T cells, assessed by CTV dilution, was suppressed when both BMDCs and co-cultures were exposed to D-mannose, but not when the BMDCs alone were loaded with OVA in the presence of D-mannose (Fig. 1I - J). The same result was obtained for the expression of the activation marker CD69 (Fig. 1K). Taken together, our findings suggest that D-mannose induces an immature, anti-inflammatory phenotype in DCs that inhibits antigen-specific T cell proliferation and activation during antigen presentation. D-mannose may also have a synergic effect on both BMDCs and T cells.

D-mannose expanded the steady-state Treg population in non-autoimmune B6 mice

In vivo, we first tested the effect of D-mannose in unmanipulated B6 mice treated for up to 9 weeks. At week 3, the frequency of circulating CD4⁺ Foxp3⁺ Treg cells was higher in D-mannose-treated mice (Fig. 2A and B), while the frequency of total CD4⁺ T cells remained unchanged (Fig. 2C). After 9 weeks of treatment, the frequency of Treg cells was increased in the spleen of D-mannose treated-mice (Fig. 2D). Moreover, the expression of Treg-associated receptors, including CD122, CD132 (the IL-2R β and γ chains, respectively), and GITR, was increased in D-mannose treated mice (Fig. 1E - G). No difference was, however, observed for the expression of CD25, the IL-2R α high affinity chain (data not shown). There was also a trend for a higher production of IL-10 in the Treg cells from D-mannose treated mice (data not shown). These results showed that D-Mannose expanded the Treg population and the expression of some of its markers under homeostatic conditions.

D-mannose treatment decreased autoimmune activation in the cGVHD model

We next investigated the effect of D-mannose in the cGVHD model, a well-established model of induced systemic autoimmunity [12]. After three weeks of pre-treatment with or without D-mannose, B6 recipients received CD4⁺ T cells from the semi-allogenic bm12 donor, and continued their treatment with or without D-mannose for another 3 weeks (Fig. 3A). Mice were sacrificed three weeks after induction, which corresponds to the peak of anti-dsDNA IgG production in this model [16]. D-mannose reduced anti-dsDNA IgG production starting 2 weeks after induction (Fig. 3B). This suppressive effect was associated with a lower number of cells in the mLN, and a similar trend was observed in the spleen (Fig. 3C). The frequency and number of splenic B cells remained unchanged (Fig. 3D - F), but their activation measured as the expression of MHC-II was decreased in D-mannose treated mice (Fig. 3G). D-mannose also decreased the frequency of germinal center (GC) B cells in both spleen and mLN, but cell numbers decreased only in

mLN (Fig. 3H - I). There was also a reduction in the number of plasma cells of mLN and a similar trend in the spleen (Fig. 3J-K).

CD4⁺ T cell activation has been a major focus of investigation in the SLE-cGVHD model [17]. D-mannose treatment did not change the frequency or number of total CD4⁺ T cells (data not shown), but alleviated CD4⁺ T cell activation measured as the frequency of CD44⁺CD62L⁻ effector memory (T_{EM}) cells (Fig. 4A - B), as well as the T_{EM} over naive CD44⁻CD62L⁺ (T_N) ratio in spleen (Fig. 4C). In addition, the frequency of follicular helper (T_{FH}) T cells and the ratio of T_{FH}/follicular regulatory (T_{FR}) in spleen were also reduced after D-mannose treatment (Fig. 4D - E). Contrary to the results observed in non-inflammatory conditions (Fig. 2), the frequency of Treg cells was not altered by D-mannose in cGVHD-induced mice (Fig. 4F). However, cGVHD expanded the frequency of Treg cells in control mice, most likely as a response to inflammation, but not in D-mannose treated mice (Fig. 3G). In addition, a higher expression of the IL-2 receptor gamma chain (CD132) was observed in the splenic Treg cells from the D-mannose treated group in both cGVHD-induced mice (Fig. 3G) and in unmanipulated mice (Fig. 2F).

Finally, we determined the effect of D-mannose on DCs by assessing their frequency and the expression of activation markers. The frequency of plasmacytoid DCs (pDCs) was comparable in D-mannose treated and non-treated mice, but there was a small expansion in the frequency of conventional dendritic cells (cDCs) in D-mannose-treated cGVHD-induced mice (Fig. 5A). In addition, the splenic cDCs from D-mannose treated mice displayed an increased expression of the mannose receptor (Fig. 5B) and a decreased expression of the activation markers CD80 and CD40 (Fig. 5C - D). Overall, these results suggest that D-mannose limits the development of autoimmune activation in cGVHD model.

D-mannose decreased the number of T_{EM} and T_{FH} cells and expanded the frequency of Treg cells in cGVHD recipients

To differentiate the effect of D-mannose on donor or recipient CD4⁺ T cells in cGVHD, we used B6.SJL as recipients, in which we can distinguish recipient CD4⁺ T cells (CD45.1-B6.SJL) from the donor CD4⁺ T cells (CD45.2⁺-bm12). D-mannose affected CD4⁺ T cells in recipient mice, first by decreasing the frequency and number of recipient-derived Tem (Fig. 6A - B) and number of recipient Tfh cells (Fig. 6C - D) in D-mannose treated mice. Moreover, D-mannose increased the frequency of recipient Treg cells (Fig. 6E - F). It should be noted that less than 1% of the donor CD4⁺ T cells recovered after cGVHD expressed Foxp3 while over 80% of these donor cells expressed a T_{EM} phenotype (data not shown). These results confirm the immunoregulatory effect of D-mannose in the cGVHD model and showed that it does not change the inflammatory phenotypes of the donor cells, but their response in recipient cells.

D-mannose treatment decreased CD4⁺ T cell activation in a B6.lpr mice

To assess the efficacy of D-mannose in a spontaneous model of lupus, we selected B6.lpr mice, a simplified model driven by FAS deficiency. Treatment was started at 4 months of age, when B6.lpr mice start to produce autoantibodies, and lasted for 10 weeks. There was an initial reduction of anti-dsDNA

IgG production, which reached significance at week 4 after treatment, but it was not sustained thereafter (Fig. 7A). There was a trend for an increase of the frequency of T_N cells (Fig. 7B) and Treg cells (Fig. 7C) by the D-mannose treatment. $CD4^+$ T cell activation, measured as CD44 expression (Fig. 7D) and the T_{FH}/T_{FR} ratio, (Fig. 7E) was reduced by the D-mannose treatment. Finally, splenic cDCs from D-mannose treated mice expressed higher levels of CD206 (Fig. 7F), which was in line with the result obtained in the cGVHD model. These results showed that the immunoregulatory effect of D-mannose is not restricted to the cGVHD induced model of lupus, but also applies to the B6.lpr model, in which autoimmunity arises from a completely different pathway.

Discussion

The purpose of this study was to evaluate whether the protective effect of D-mannose that has been reported for type 1 diabetes, airway inflammation [8], and in an induced model of arthritis [11] could be extended to models of systemic autoimmunity. D-mannose treatment has not been tested in lupus, either *in vitro* or *in vivo*. Furthermore, the potential involvement of CD206, the mannose receptor, in the regulation of immune cells in SLE is largely unknown. CD206 is a marker of M2 macrophages, which are generally associated with anti-inflammatory phenotypes. Activated $CD206^+$ M2b macrophages have been shown, however, to be pathogenic in a spontaneous model of lupus nephritis [18] and they represent the largest myeloid infiltrates in human lupus nephritis [19, 20]. On the other hand, CD206 expression on macrophages was induced by a therapeutic treatment with mesenchymal stem cells in an IL-6 dependent manner in the B6.lpr model [21]. Whether the expression of CD206 on these pro- or anti-inflammatory macrophages is necessary for their function is unknown.

We started our study with two cell types in which D-mannose has shown to modulate functions, DCs and Treg cells. Blocking the MR modulated the differentiation, maturation, and functions of DCs resulting in a reduced severity of induced autoimmune arthritis [11]. Moreover, deleting or blocking CD206 on DCs decreased the proliferation and cytotoxic activity of urine $CD8^+$ T cells *in vitro* [10]. The expression of surface molecules on DCs is dynamic and changes according to tissue localization and maturation state. Most immature DCs are characterized by expression of molecules associated with antigen uptake such as CD32b and CD64, but also CD206, which is an endocytic receptor. With activation, mature DCs lose their capacity for antigen engulfment, and acquire molecules necessary for stimulation of T lymphocytes, such as CD40, CD80, and CD86 [22]. In this study, we showed that D-mannose enhanced the expression of CD206 on cDCs both *in vitro* (BMDC) and *in vivo* (cGVHD and lupus-prone B6/lpr model). In addition, CD32b and CD64 expression remained high in D-mannose treated BMDCs in spite of LPS stimulation. These results indicate that D-mannose maintains DCs in an immature state despite activation, which is consistent with the finding that cross-linking CD206 induced anti-inflammatory DCs [23]. Here we showed that this result can be achieved directly with D-mannose.

Moreover, D-mannose treatment inhibited DC activation as shown *in vitro* by the reduced expression of pro-inflammatory cytokines and in the cGVHD model by a lower expression of the activation markers

CD40 and CD80, which mediate interactions with T cells. Accordingly, D-mannose inhibited antigen specific proliferation and activation in CD4⁺ T cells in a co-culture assay. This inhibitory effect could not be detected when antigen uptake occurred in the presence of D-mannose, but without D-mannose in the co-culture medium. This result could be interpreted in two manners: first, the presence of D-mannose may be critical to maintain DCs in an immature state during antigen presentation; or second, D-mannose may directly induce the differentiation of Treg cells, which may synergize with immature DCs to suppress T cell proliferation and activation.

D-mannose promoted the expansion of Treg cells from naive CD4⁺ T cells in spontaneous type 1 diabetes as well as in an TCR transgenic model of induced airway inflammation [8]. We confirmed this effect of D-mannose in unmanipulated B6 mice, and further showed an induction of markers commonly associated with regulatory functions in Treg cells, namely, GITR, CD132, and CD122. In the cGVHD model, D-mannose upregulated the expression of CD132 on Treg cells, but it did not change Treg frequency or number. There was a trend for an increased frequency of Treg cells in D-mannose treated B6.lpr mice, which may suggest that the induction of regulatory markers of Tregs may be more critical than the expansion of Treg number. Lupus cytokine milieu promotes the expansion of non-functional Treg cells [24] that may not respond to D-mannose as efficiently as control Treg cells. In support of this hypothesis, we showed that the frequency of Treg cells is higher in cGVHD-induced mice than in controls.

Nonetheless, we have shown that D-mannose treatment reduced autoantibody production in the cGVHD model of systemic autoimmunity as well as, at least at early age, in B6.lpr mice. This was associated with a reduction of CD4⁺T cell differentiation into T_{EM} cells and T_{FH} cells, as well as the T_{EM}/T_N cells and T_{FH}/T_{FR} ratios. These effector T cell phenotypes are strongly associated with lupus pathogenesis in both mouse models and human patients [25]. Donor CD4⁺ T cells are both necessary and sufficient for cGVHD induction, but no effect of D-mannose could be detected in donor derived CD4⁺ T cell subpopulations, including Treg, T_{EM} nor T_{FH} cells. However, there were significantly less recipient-derived T_{EM} and T_{FH} cells in mesenteric lymph nodes of D-mannose treated group. Interestingly, more than 90% of Treg cells were of recipient origin, which was much higher compared with the percentage of T_{EM} and T_{FH} cells of recipient origin. Notably, the effect of D-mannose treatment was overall stronger in the mLN than in the spleen. The intraperitoneal induction of cGVHD combined to the oral delivery of D-mannose may be responsible for this tissue distribution. The underlying mechanism of the preferential effects on gut-associated recipient T cells warrants further investigation.

Conclusions

We have shown an immunoregulatory effect of D-mannose in an induced lupus model, which was associated with the expansion of Treg cells, the induction of immature conventional dendritic cells and the downregulation of effector T cells activation. D-mannose had a more potent effect in type 1 diabetes and OVA-induced airway inflammation [8] than what we observed here in induced and spontaneous models of lupus. This may be related to a relatively reduced role of Treg cells in lupus as compared to the

two other types of autoimmune pathology, in which D-mannose beneficial effect was associated with a strong expansion of the Treg population. This was not observed in the lupus models. Our findings warrant further exploration of the basic immunological mechanisms and potential clinical applications of D-mannose.

Abbreviations

D-man, D-mannose; cGVHD, chronic graft-versus-host-disease; Treg, regulatory T cells; GC, germinal center; T_{FH} , follicular helper T cells; T_{FR} , T follicular regulatory cells; T_N , Naïve T cells; Teff, effector T cells; OXPHOS, oxidative phosphorylation; OCR, oxygen consumption rate; ECAR, extra cellular acidification rate; FAO, fatty acid oxidation; SLE, systemic lupus erythematosus.

Declarations

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

HW and XT designed, performed experiments, and wrote the manuscript, GA and WL contributed reagents and participated to experimental design and data interpretation, SY contributed to experimental design and data interpretation, LM conceived the study, contributed to experimental design and data interpretation, and wrote the manuscript.

Ethics declarations

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Animal Welfare Act and the National Institutes of Health guidelines for the care and use of animals in biomedical research. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Florida (IACUC # 201909466).

Consent for publication

Not applicable.

Competing interests

The authors have declared no conflicting interests.

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Figures

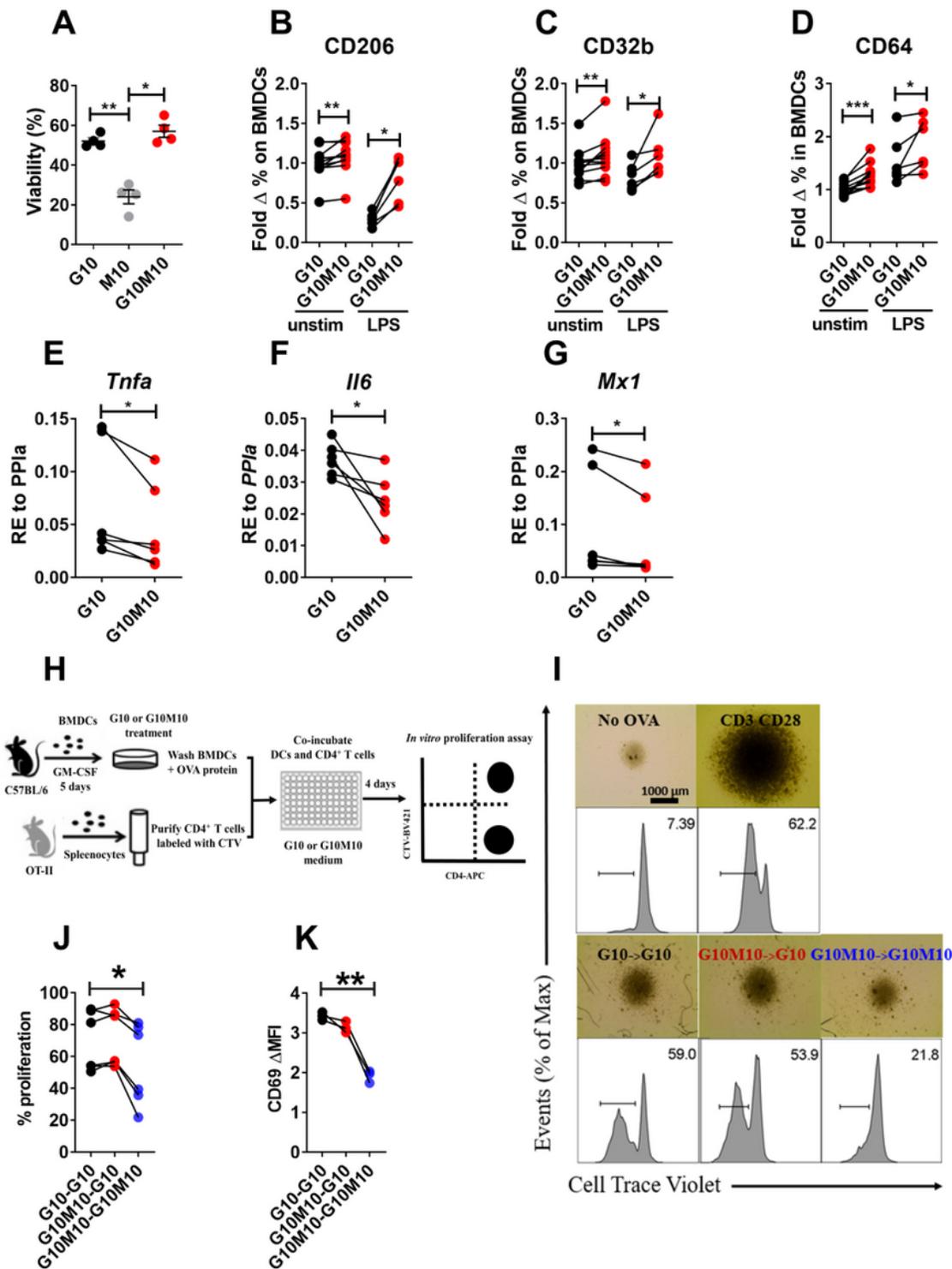


Figure 1

Figure 1

D-mannose inhibits BMDCs activation in vitro and BMDC-induced antigen-specific T-cell responses in vitro. BMDCs from B6 mice were cultured with G10, M10, or G10M10 for 24 h with or without LPS. Cell viability (A) and surface marker expression (B-D) were analyzed by flow cytometry. Gene expression in stimulated cells for *Tnfa*, *Il6*, and *Mx1* (E-G). H. B6 BMDCs were treated with G10 or G10M10 medium for 24 h, then loaded with OVA protein and co-incubated with OT-II CD4+ T cells in G10 or G10M10 coculture

medium for 4 d. At d5, proliferation (I and J) and CD69 expression (D) were measured in CD4⁺ T cells by flow cytometry comparing 3 conditions: G10 for both BMDCs and co-culture (G10 – G10), G10M10 BMDCs and G10 co-culture (G10M10 – G10), or G10M10 for both BMDCs and co-culture (G10M10 – G10M10). Mean \pm S.E.M of n = 4 - 7 per group from 2 - 3 separate experiments compared with t tests. *: p < 0.05, **: p < 0.01, ***: p < 0.001.

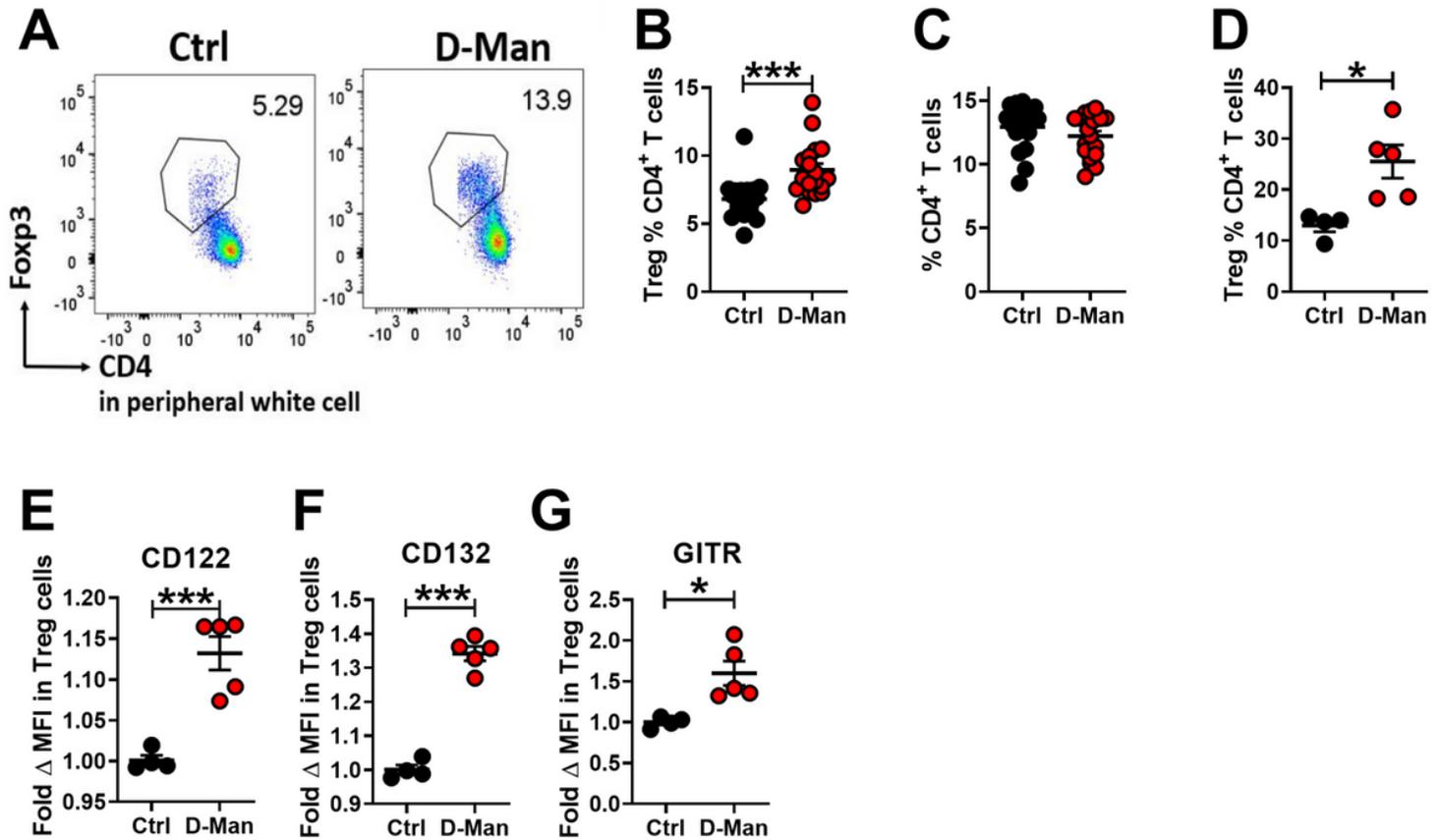


Figure 2

Figure 2

D-mannose expanded the steady-state Treg population. B6 mice were treated with or without D-mannose in drinking water. Phenotypes were analyzed at week 3 in the peripheral blood and week 9 in the spleen. A. Representative FACS plots of Treg cells in CD3⁺-gated PBMCs. Frequency of Treg cells (B) and CD4⁺ T cells (C) in CD3⁺ PBMCs. n = 16 per group from 2 separate experiments compared with t tests. D. Frequency of Treg cells in CD4⁺ splenocytes. CD122 (E), CD132 (F) and GITR (G) MFI on Treg cells expressed as fold change relative to the Ctrl group. Mean \pm S.E.M of n = 4 - 5 per group compared with t tests. *: p < 0.05, ***: p < 0.001.

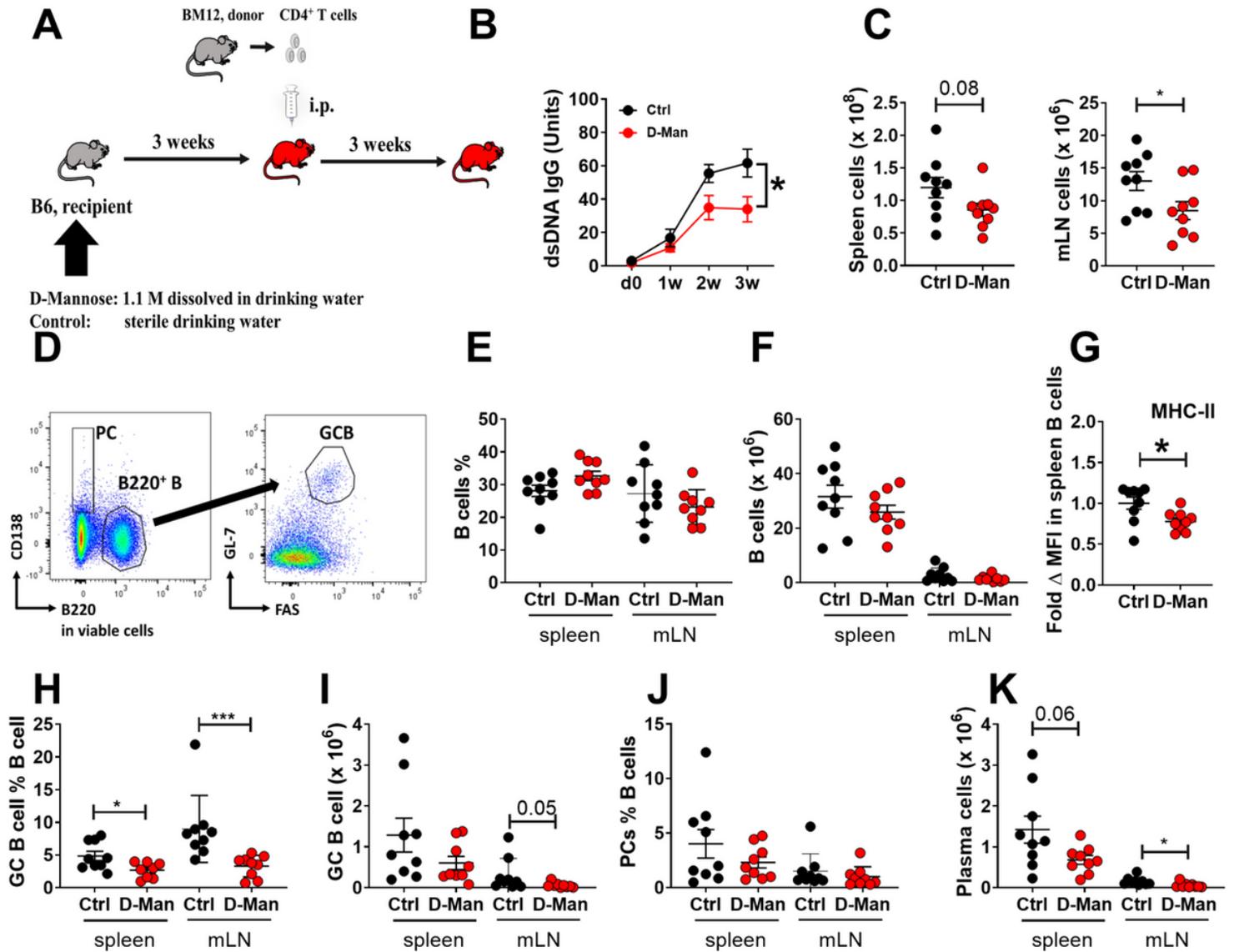


Figure 3

Figure 3

D-mannose reduced autoantibody production, lymphoid expansion as well as B cell differentiation in the cGVHD model. A. Experimental design. B. Serum anti-dsDNA IgG. C. Representative FACS plots of B cells showing the GC B cell and plasma cell gates. Spleen and mLN cell numbers. Frequency (E) and number (F) of B cells in spleen and mLN. G. MHC-II MFI on splenic B cells expressed as fold change relative to the Ctrl group. Frequency and number of GC B cells (H and I) and plasma cells (J and K) in spleen and mLN. Mean \pm S.E.M of n = 9 - 12 per group from 2 - 3 cohorts compared with t tests. *: p < 0.05.

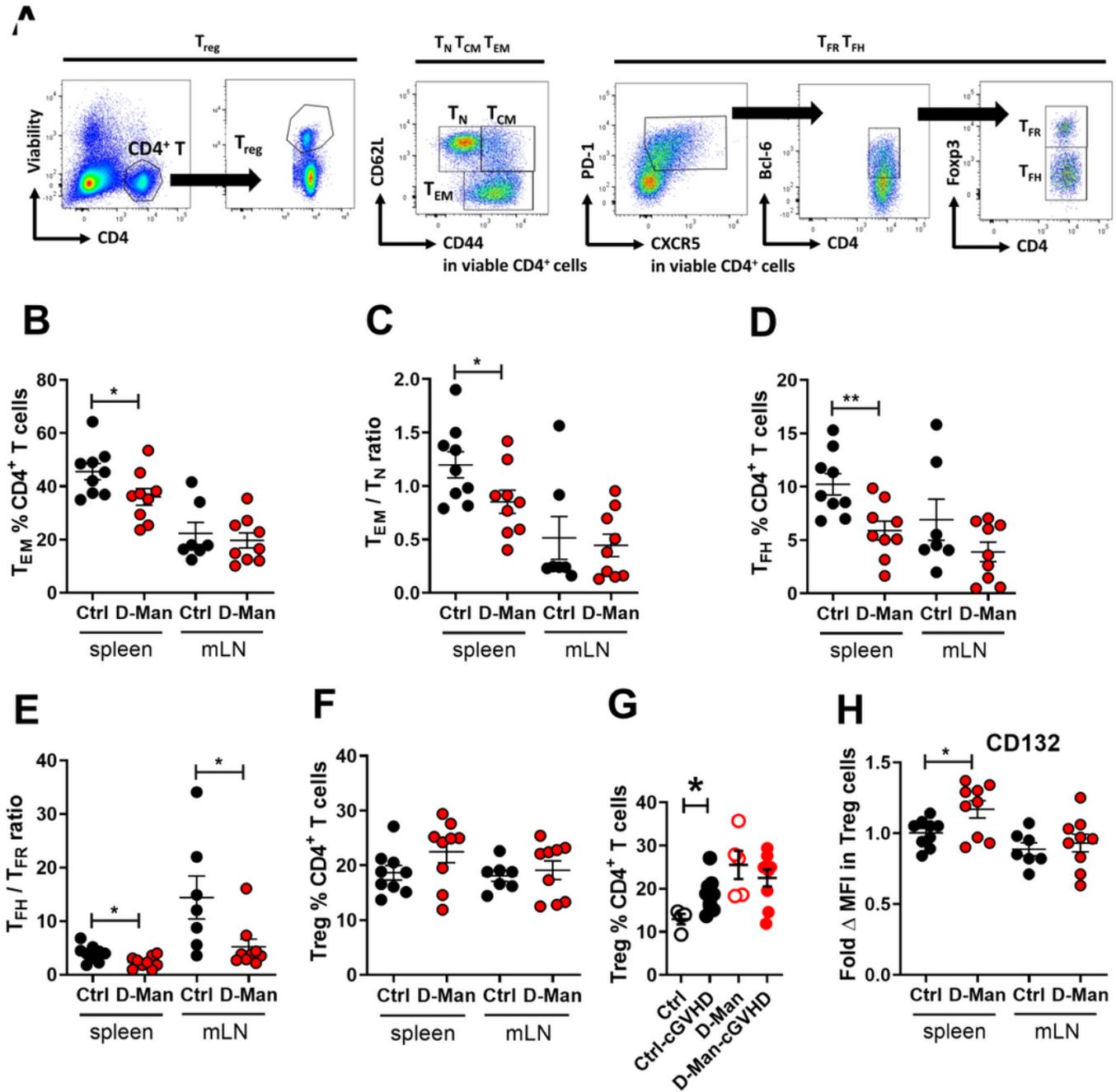


Figure 4

Figure 4

D-mannose reduced the expansion of effector CD4⁺ T cells in the cGVHD model. A. Representative FACS plots of CD4⁺CD44⁺CD62L⁺ T_N, CD4⁺CD44⁺CD62L⁻ T_{EM}, CD4⁺PD-1⁺CXCR5⁺Bcl-6⁺Foxp3⁺ T_{FR} and CD4⁺PD-1⁺CXCR5⁺Bcl-6⁺Foxp3⁻ T_{FH} cells, and CD4⁺Foxp3⁺ T_{reg} cells 3 weeks after cGVHD induction. B. Frequency of T_{EM} cells. C. T_{EM}/T_N ratio. D. Frequency of T_{FH} cells. E. T_{FH}/T_{FR} ratio. F. Frequency of Treg cells. G. Comparison of the effect of D-mannose on splenic Treg cell frequency in unmanipulated

mice (Ctrl) or cGVHD-induced mice. H. CD132 MFI on Treg cells expressed as fold change relative to the Ctrl group. Mean \pm S.E.M of n = 9 per group from 2 - 3 cohorts compared with t tests. *: p < 0.05, **: p < 0.01.

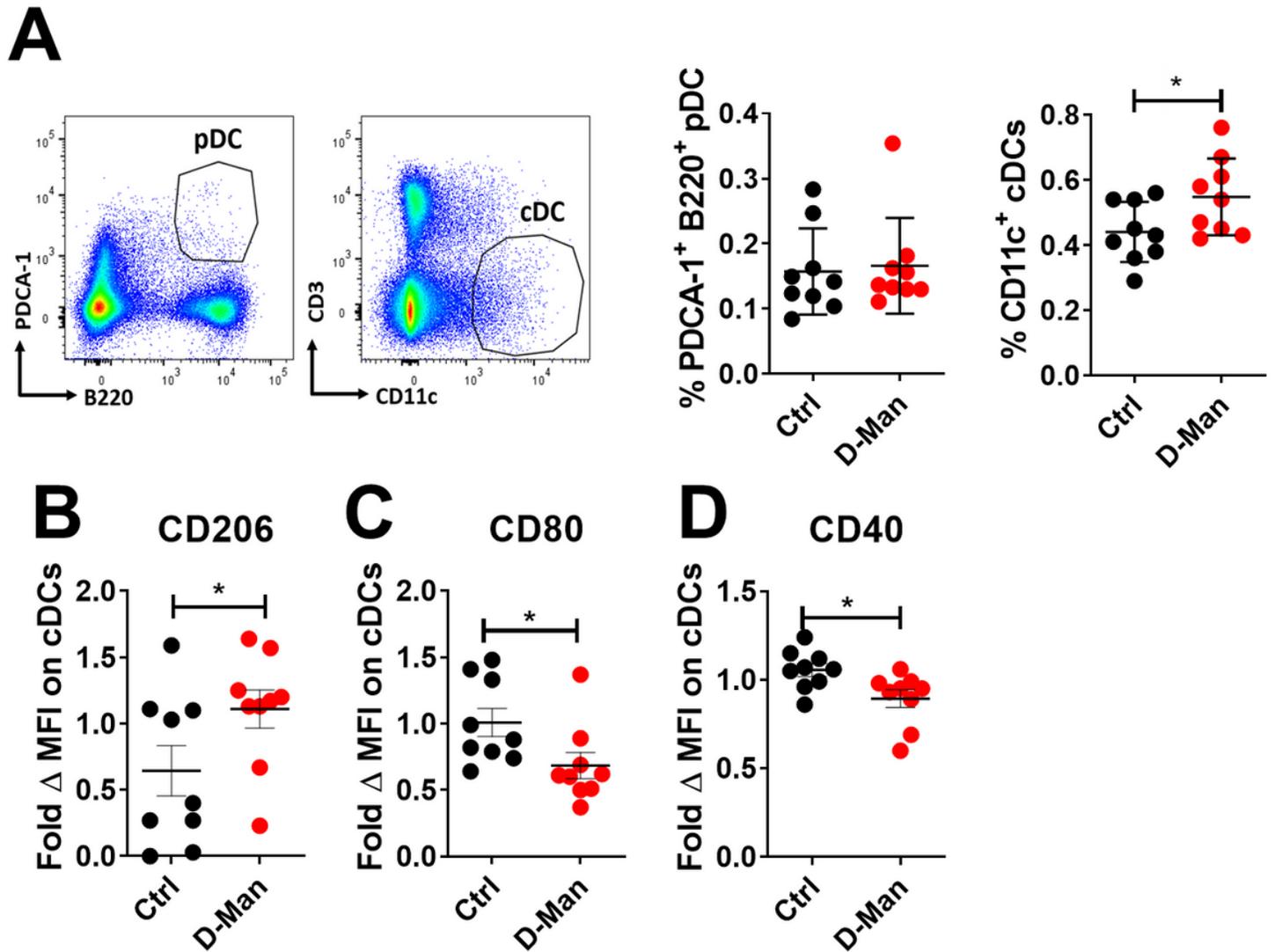


Figure 5

Figure 5

D-mannose reduced cDCs activation. A. Representative FACS plots showing B220+PDCA-1⁺ pDC and CD11c+CD3⁻ cDC gating, as well as their number and frequency in the spleen of cGVHD-induced mice. CD206 (B), CD80 (C), and CD40 (D) MFI on these cDCs expressed as fold change relative to the Ctrl group. Mean \pm S.E.M of n = 9 per group from 2 - 3 cohorts compared with t tests. *: p < 0.05, **: p < 0.01.

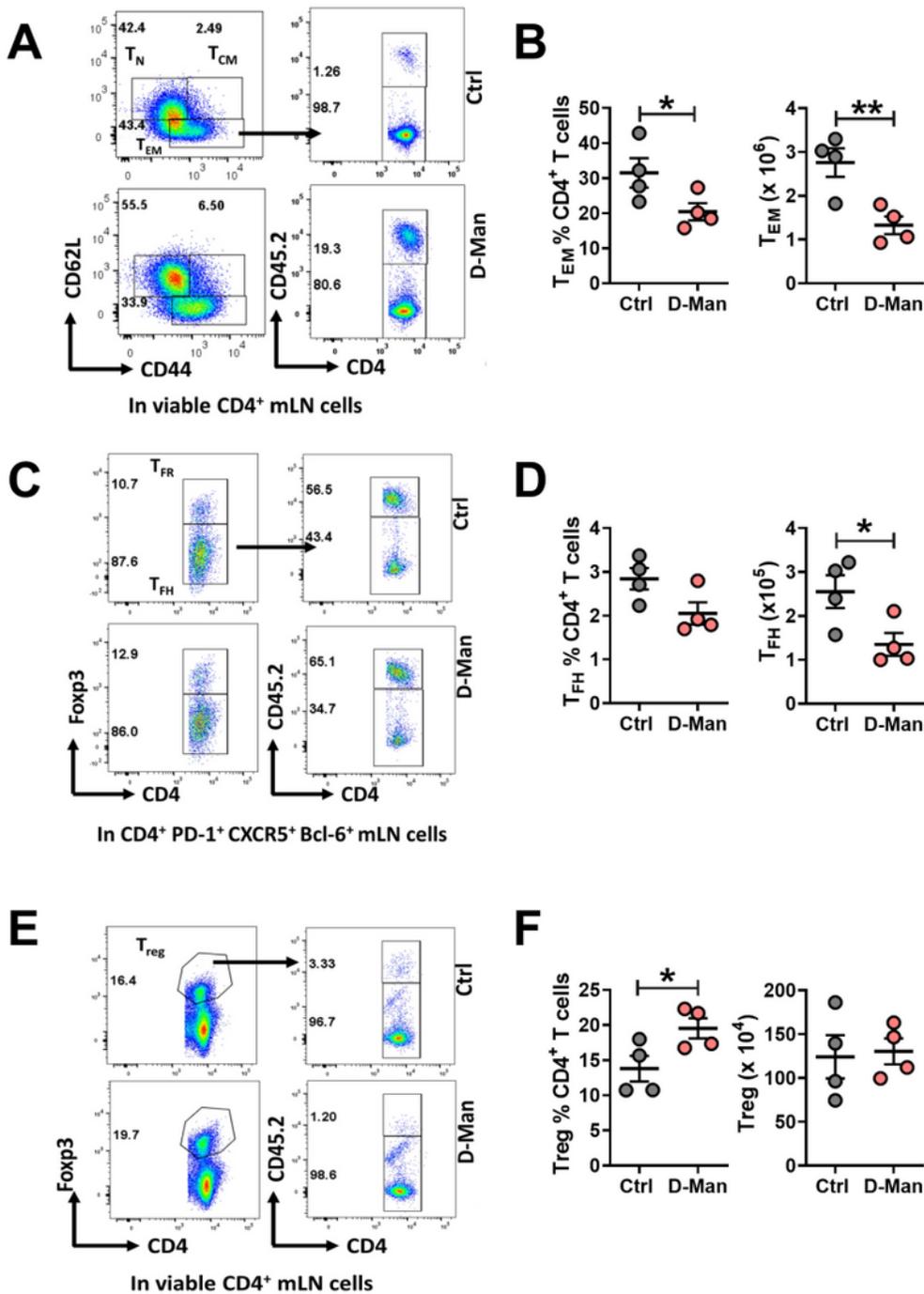


Figure 6

Figure 6

D-mannose decreased the number of TEM and TFH cells and increased the frequency of Treg cells in recipient mice in the cGVHD model. Frequency and numbers of recipient Tem (A-B), Tfh (C-D) and Treg (E-F) cells in the mLN. Representative FACS plots of donor (CD45.2⁺) and recipient (CD45.2⁻) cells are shown on the left. Mean \pm S.E.M of n = 4 per group compared with t tests. *: p < 0.05, **: p < 0.01.

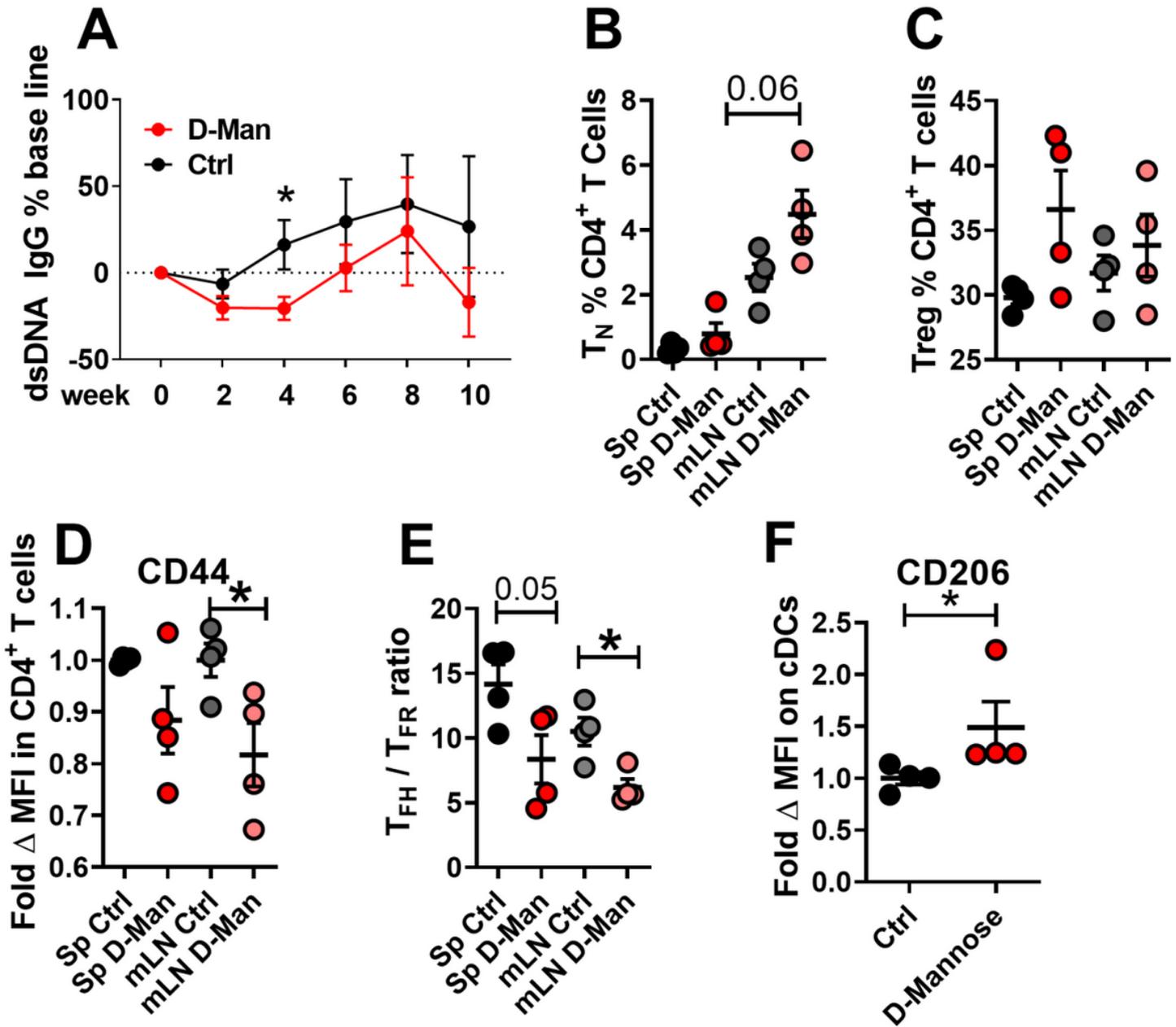


figure 7

Figure 7

D-mannose ameliorates lupus phenotypes in B6/lpr mice. Four month old B6.lpr mice were treated with D-mannose in drinking water for 10 weeks. A. Serum anti-dsDNA IgG. Frequency of T_N (B) and Treg cells (C). D. CD44 MFI on CD4⁺ T cells. E. T_{FH}/T_{FR} ratio in spleen and mLN. F. CD206 MFI on spleen cDCs. Mean \pm S.E.M of n =4 per group compared with t tests. *: p < 0.05.

Supplementary Files

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- [SupplementalTable1.docx](#)