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Deletion of CD24 Impairs Development of Heat Shock Protein gp96–Driven Autoimmune Disease through Expansion of Myeloid-Derived Suppressor Cells

Jessica E. Thaxton,* Bei Liu,* Pan Zheng,† Yang Liu,† and Zihai Li*

CD24 binds to and suppresses inflammation triggered by danger-associated molecular patterns such as heat shock proteins (HSPs) and high-mobility group box 1. Paradoxically, CD24 has been shown to enhance autoimmune disease. In this study, we attempt to reconcile this paradox by deletion of CD24 (24KO) in a lupus-like disease model driven by forced expression of HSP gp96 at the cell surface (transgenic mice [tm]). As expected, tm24KO mice showed increased CD11c+ dendritic cell activation coupled to a significant increase in dendritic cell–specific IL-12 production compared with tm mice. However, tm24KO mice showed less CD4 T cell activation and peripheral inflammatory cytokine production in comparison with tm mice. We characterized an enhanced immune suppressive milieu in tm24KO mice distinguished by increased TGF-β and greater regulatory T cell–suppressive capacity. We found greater absolute numbers of myeloid-derived suppressor cells (MDSCs) in tm24KO mice and showed that the Ly6C+ MDSC subset had greater suppressive capacity from tm24KO mice. Deletion of CD24 in tm mice led to diminished lupus-like pathology as evidenced by anti-nuclear Ab deposition and glomerulonephritis. Finally, we show that expanded MDSC populations were mediated by increased free high-mobility group box 1 in tm24KO mice. Thus, the deletion of CD24 in an HSP-driven model of autoimmunity led to the unexpected development of regulatory T cell and MDSC populations that augmented immune tolerance. Further study of these populations as possible negative regulators of inflammation in the context of autoimmunity is warranted. The Journal of Immunology, 2014, 192: 5679–5686.
tory suppression. In the context of autoimmune disease, we determined decreased lupus-like clinical manifestations due to the presence of MDSC and the consequent decreased inflammatory atmosphere. Finally, we show that elevated levels of free HMGB1 mediate MDSC infiltration in the absence of CD24 in an autoimmune disease context.

Materials and Methods

Mice

CD24<sup>−/−</sup> mice, which were produced using embryonic stem cells from C57BL/6 mice, have been described (23). Membrane-bound gp96 transgenic mice (tm; on a pure C57BL/6 background) were developed as described previously (13). CD24KO and tm mice were backcrossed for eight consecutive generations (tm24KO). Mice used for experiments were aged 6–12 mo unless otherwise indicated. The Division of Laboratory Animal Resources of the Medical University of South Carolina maintained mice according to established guidelines.

Ab and reagents

All flow cytometry and fluorescent microscopy Abs were purchased from eBioscience unless otherwise indicated. CD11c, CD40, CD44, CD80, CD86, CD69, CD25, CD11b, Gr1, Ly6C, CD19, CD138, CD4 (BD Biosciences), and CD8 (BD Biosciences) were used for extracellular staining. BrdU (BD Biosciences), IFN-γ, TNF-α, IL-12, IL-10, and Foxp3 were used for intracellular staining, and Foxp3 Buffer was used for permeabilization (eBioscience).

Cellular preparation, stimulation, and isolation

Spleens and lymph nodes were harvested in RPMI 1640 media supplemented with 10% FBS. Single-cell suspensions were obtained by tissue dissociation with glass slides and subsequent lysis of RBCs. For direct flow cytometry, cells were kept on ice and immediately placed in PBS 2% FBS for staining, which were fluorescein-conjugated Abs. For restimulation, cells were treated with or without PMA/ionomycin (0.2 and 1 μg/ml, respectively; Sigma-Aldrich) for 4–6 h with brefeldin A (BFA; BD Biosciences), and extracellular/intracellular flow cytometry Ab staining was performed. BrdU staining protocols were followed per the manufacturer’s instructions (BD Biosciences). PBMCs were obtained by harvesting blood into heparinized tubes. Blood was stained at room temperature for 20 min with fluorescent-conjugated Abs, then washed, and lysed with ACK. Cells were fixed or permeabilized for intracellular staining. For Western blot, cells were lysed with RIPA buffer on ice for 1 h.

Cell cultures and Greiss reaction

Cells were cultured in RPMI 1640 supplemented with 10% FBS and antibiotics. For TGF-β ELISA, supernatants were collected after 24 h of culture. For Greiss reaction, cells were plated at 1 x 10⁶ cells/well in 96-well plates and treated with or without 1 μg/ml LPS (Sigma-Aldrich) for 24 h. Supernatants were harvested, and Greiss reaction was performed per the manufacturer’s instructions (Life Technologies).

BrdU and CFSE assays

Mice were given BrdU (0.8 ng/ml) in drinking water for 3 consecutive d. CFSE was loaded into CD8 T cells per the manufacturer’s instructions (Invitrogen) and plated at ratios of 1:1 and 10:1 (T/suppressor cells). CD8 T and suppressor cell subsets were isolated from single-cell splenics suspensions via magnetic bead sort per the manufacturer’s instructions (Treg Kit and MDSC Kit; Miltenyi Biotec). The 1:1 ratios were plated at 1 x 10⁶ cells in 96-well plates coated with anti-CD3 (eBioscience). On day 3 of coculture, cells were washed and CFSE dilution was read via flow cytometer.

ELISA and Western blot

ELISA was used to detect TGF-β (R&D Systems), IL-12p40 (BD Biosciences), and IgM, IgA, IgG1, IgG3, IgG2b, and IgG2c (Southern Bio-technology Associates) per the manufacturer’s protocol. Serum Western blot for HMGB1 was performed on diluted sera samples (1:10), and Coomassie blue stain of gel was used to detect albumin band as loading control. Lysates and sera were measured by BSA assay (Thermo Fisher). Denatured proteins were run on 10% SDS gel, and primary proteins were blotted overnight at 4°C. Primary Ab for HMGB1 was obtained from Cell Signaling Technology. Anti-rabbit HRP secondary Ab was used for protein detection.

Chemotaxis assay

Recombinant HMGB1 was obtained from HMGBioTech. HMGB1 was rigorously tested by HMGBioTech and determined to be LPS free. An i.p. injection of 100 ng/mouse in sterile PBS was administered and PBMCs were assessed 1 h pre- and 4 h postinjection, respectively.

Immunofluorescence detection of glomerulonephritis and clinical evaluation of the severity of kidney disease and detection of anti-nuclear Abs

Kidney sectioning, immunofluorescent staining, H&E, anti-nuclear Ab (ANA) detection, and clinical assessments were performed and assessed as previously described (24). For ANA and glomerulonephritis (GN) immunofluorescence, we used a five-point scale for assessment of clinical disease in which fluorescent intensity was graded as 1 (negative staining), 2 (dim staining), 3 (dim-moderate staining), 4 (moderate-bright staining), or 5 (bright staining).

Statistics

Student t test two-tailed paired statistics were performed. Paired tests were used where age-matched controls were measured unless otherwise specified. The p values < 0.05 were considered significant. Mice analyzed were n = 3 pairs or more for all experiments.
Results
Loss of CD24 in tm mice leads to increased DC activation

We previously engineered transgenic mice to express gp96 at the cell membrane (tm) and demonstrated that these mice developed lupus-like disease 4 mo after birth (13). We further showed that IL-12 and CD11c+ DCs contributed to disease development (14). CD24 is a critical receptor for DAMP HMGB1 on DCs, and in the absence of CD24, DCs show amplified inflammatory activity to DAMPs upon exogenous stimulation (8). To address the role of CD24 in response to DAMPs released due to endogenous inflammation, we crossed CD24KO mice to tm mice (tm24KO). At 6 mo after birth, we assessed CD11c+ cell populations and noted that mice from tm24KO

![Image](https://example.com/image1.png)

FIGURE 2. Decreased T cell activation, proliferation, and cytokine production in tm24KO mice. The tm and tm24KO mice were subjected to flow cytometric analysis. CD4+ single-positive populations were gated. (A) Splenocytes from 6-mo tm or tm24KO mice were surface stained for CD69+ and intracellular stained for BrdU after 3 d of BrdU water treatment. Data of one representative pair of mice from four pairs of mice are shown. Graph depicts statistical significance for CD4/CD69+ and CD4/CD69+/BrdU+ cells. (B) Splenocytes or mln cells were incubated with PMA/ionomycin in the presence of BFA for 4–6 h. Intracellular stains of IFN-γ (shown) and TNF-α were performed. Top panel shows CD4-specific IFN-γ production. Bottom panel is gated on CD4+ single-positive cells and assessed for CD44/IFN-γ double-positive populations. Data shown are one pair of mice representative of four pairs of mice. Graph (right panel) depicts statistical significance for CD4/TNF-α+ or CD4/IFN-γ+ splenocytes and mln cells. (C) PBMCs were incubated with PMA/ionomycin in the presence of BFA for 4–6 h. Intracellular stains of IFN-γ (shown) and TNF-α were performed. Data of one representative pair of mice from five pairs of mice are shown. Graph depicts statistical significance for CD4/TNF-α+ or CD4/IFN-γ+ PBMCs. *p < 0.05.
groups consistently showed greater CD11c+ populations as a percentage of splenocytes (Fig. 1A). We next assessed DC activation as measured by CD80, CD86, and CD40. We found that PBMC DC populations from tm24KO had significantly increased expression as measured by mean fluorescence intensity (MFI) of CD80 and CD86 as compared with tm levels (Fig. 1B). We next asked whether tm24KO peripheral blood DCs (PB-DCs) showed enhanced IL-12 production compared with tm mice. Direct ex vivo data show that PB-DCs from tm24KO mice have a higher MFI of IL-12 than PB-DCs from tm mice (Fig. 1C). We further quantified levels of serum IL-12p40 and noted that enhanced DC activity in tm24KO mice correlated to significantly elevated levels of IL-12p40 in tm24KO mice as compared with tm mice (Fig. 1D).

**Decreased inflammatory CD4 T cells in tm24KO mice**

IL-12 is an inducer of Th1 differentiation and leads to enhanced T cell proliferation and IFN-γ production (25). We assessed CD4/CD8 populations in tm and tm24KO mice and did not note a difference between these populations (data not shown). We further investigated CD4 T cells by measurement of early activation marker CD69. We found that splenic tm24KO CD4 T cells expressed less CD69 than tm CD4 T cells. To determine whether tm24KO CD4 T cells were truly less active than tm CD4 T cells, we fed mice BrdU water and assessed BrdU incorporation after 3 d. We found that CD4/CD69+ populations of tm24KO mice showed decreased BrdU incorporation as compared with tm mice, and this effect was significant in splenocytes. These results indicate low CD4 T cell proliferation in tm24KO mice (Fig. 2A). To quantify inflammatory potential of T cells, we isolated and stimulated (PMA/ionomycin) mixed lymphocytes from tm and tm24KO mice. We found increased IFN-γ and TNF-α (data not shown) production from mesenteric lymph nodes (mLN) of tm mice as compared with tm24KO mice (Fig. 2B, top panel). We further assessed CD4 T cell activation in spleens and mLN by analysis of CD44 expression. We determined that IFN-γ (Fig. 2B, bottom panel) and TNF-α production (data not shown) were produced by CD44high CD4 T cell subsets in tm and tm24KO mice (Fig. 2B). Though not significant, tm24KO mice consistently showed less inflammatory cytokine production from CD44high CD4 T cell subsets. Due to enhanced TNF-α and IFN-γ in lymph nodes that approached significance, we focused on T cells in circulation. We performed stimulation of CD4 T cells from peripheral blood of tm and tm24KO mice. Production of TNF-α and IFN-γ were increased in tm CD4 T cells as compared with tm24KO CD4 T cells (Fig. 2C). Therefore, it is likely that enhanced activation of T cells led to increased peripheral migration and subsequent inflammatory surveillance in tm mice.

**Hallmarks of anti-inflammatory immunity**

To better understand the cause of decreased CD4 T cell activation in tm24KO mice, we assessed parameters of anti-inflammatory immunity. The cytokine TGF-β is tied to activity of immune-suppressive populations such as Tregs and MDSCs (26, 27). We measured active TGF-β secretion from PBMCs cultured 24 h or from serum. We found significantly increased active TGF-β in culture supernatants and sera from tm24KO mice compared with tm mice (Fig. 3A). To further investigate these data, we isolated and counted absolute numbers of Tregs from tm or tm24KO mouse spleens and found significantly increased numbers of Tregs in tm24KO mice (Fig. 3B). We next directly evaluated the role of Tregs in tm24KO mice as compared with tm mice. IL-10 is a hallmark anti-inflammatory cytokine associated with Treg activation and suppression of inflammatory immunity (28). CD25, the high-affinity IL-2R, is a mark of Treg activation indicative of active IL-2 signaling and subsequent active Treg suppression (29). Compared to tm mice, Tregs from tm24KO mice displayed significantly increased ratios of CD25+ when measured from total Foxp3+/CD4+ Tregs (Fig. 3C, top panel; *p < 0.05; tm 0.56 ± 0.03; tm24KO, 0.77 ± 0.04). Further, intracellular cytokine stain for IL-10 production showed increased IL-10 from splenic Tregs in tm24KO mice compared with tm mice (*p < 0.05; tm 1.2 ± 0.7; tm24KO, 7.8 ± 2.1; Fig. 3C, bottom panel). CFSE suppression assay at a ratio of 10:1 showed increased suppression from tm24KO Tregs compared with tm Tregs (*p < 0.05; tm, 1261 ± 271; tm24KO, 1945 ± 174; Fig. 3D). Taken together, increased TGF-β, IL-10, and active Tregs indicate an enhanced regulatory immune axis in tm24KO mice as compared with tm mice.

**tm24KO mice have increased MDSC populations**

We wished to further understand the driving force behind increased suppressive immunity in tm24KO mice as compared with tm mice. Decreased naïve T cell activation, enhanced Treg activity, and increased TGF-β signaling are hallmarks of MDSC activation (10, 30). Furthermore, reports demonstrate that HMGB1 has the potential to drive enrichment of granulocytic and MDSC populations (11, 31). Due to loss of HMGB1 receptor CD24 in tm24KO mice, we wished to assess a potential contribution of MDSC populations in these mice. Absolute numbers of splenocytes indicated a signifi-

**FIGURE 3.** Measurement of Treg-associated suppressive parameters in tm and tm24KO mice. (A) Averages of active TGF-β measured by ELISA from 24-h PBMC-cultured supernatants (three pairs of mice) or serum (six pairs of mice). (B) Tregs from spleens of tm or tm24KO mice were isolated and counted. Graphs are the averages for three pairs of mice. (C) Spleenocytes or mLN single-cell suspensions were extracellularly stained for CD4/CD25 (top panel). Spleenocyte single-cell suspensions were incubated with PMA/ionomycin in the presence of BFA for 4–6 h. Cells were extracellularly stained for CD4/CD25, and intracellular staining for Foxp3/IL-10 was performed. Bottom panel is gated on Foxp3+/CD4+ cells. Spleen data of one representative pair of mice from three pairs of mice are shown. (D) CD8 T cells and Tregs were isolated from tm or tm24KO spleens. CD8 T cells were labeled with CFSE and plated at a ratio of 10:1 with strain-specific Tregs on CD3/CD28-coated plates. CFSE dilution was analyzed by flow cytometry 72 h later. Representative histograms from three separate experiments are shown. Data are presented as average MFI of CFSE. *p < 0.05.
cant increase in the CD11b+ population in tm24KO mice as compared with tm mice (Fig. 4A). We used flow cytometry to carefully assess the lineage of increased CD11b+ cells. We gated myeloid and lymphoid cells and looked at CD11b+/Gr1+ status. Both CD11b+/Gr1<sup>high</sup> and Gr1<sup>dim</sup> populations were increased in spleens of tm24KO mice when compared with tm mice. Further gating of the CD11b/Gr1 populations distinguished a heterogeneous population of cells defined by Ly6C<sup>high</sup>/Gr1<sup>low</sup> and Ly6C<sup>low</sup>/Gr1<sup>high</sup> expression. Both populations were increased in tm24KO mice (Fig. 4B). We next evaluated these populations in blood taken from tm or tm24KO mice. We found significantly increased percentages of MDSCs in circulation (PBMC) in tm24KO mice as compared with tm mice. Gating of these cells indicated both Ly6C<sup>high</sup>/Gr1<sup>low</sup> and Ly6C<sup>low</sup>/Gr1<sup>high</sup> were significantly increased in tm24KO mice as compared with tm mice (Fig. 4C).

We next measured the suppressive capacity of MDSCs between tm and tm24KO mice. Ratios of 1:1 and 10:1 were plated for Ly6G<sup>+</sup> and Ly6C<sup>+</sup> MDSC subsets. CFSE dilution of effector cells was measured by MFI. The Ly6C<sup>+</sup> subset from tm24KO mice showed greater suppressive capacity of effector cell proliferation than the subset from tm mice (p < 0.05; tm, 1075 ± 619; tm24KO, 1850 ± 232; Fig. 4D). To further measure Ly6C<sup>+</sup> subset suppression, we used Greiss reaction to assess nitrite release in response to LPS stimulation (32). Ly6C<sup>+</sup> MDSCs from tm24KO mice showed significantly increased nitrite production in response to LPS compared with subsets from tm mice (Fig. 4E).

**FIGURE 4.** Characterization of MDSC populations in tm and tm24KO mice. The tm and tm24KO mice were subjected to flow cytometric analysis. Extracellular CD11b<sup>+</sup>/Gr1<sup>+</sup> single-positive populations were gated, except for (D). (A) Spleen cells were counted, and total number of CD11b<sup>+</sup>/Gr1<sup>+</sup> cells was calculated. Graph depicts averages from three pairs of mice. Splenic (B) or PB (C) CD11b<sup>+</sup>/Gr1<sup>high</sup> (red) and CD11b<sup>+</sup>/Gr1<sup>dim</sup> (blue) cells are gated, and histogram represents Ly6C<sup>+</sup> dim (red) or high (blue) staining of these populations. (D) CD8 T cells and Ly6G<sup>+</sup> and Ly6C<sup>+</sup> cell fractions were isolated from tm or tm24KO spleens. Effector cells were labeled with CFSE and plated at ratios of 1:1 and 10:1 with strain-specific MDSC subsets. CFSE dilution was analyzed by flow cytometry 72 h later. Representative histograms from three separate experiments are shown. Data are presented as average MFI of CFSE. (E) Ly6G<sup>+</sup> and Ly6C<sup>+</sup> cell fractions were isolated from tm or tm24KO spleens, and 1 × 10<sup>5</sup> cells were plated with or without 1 µg/ml LPS for 24 h. Greiss reaction was performed on cell supernatants. *p < 0.05.

Presence of MDSCs reduces clinical manifestations of lupus-like disease

Our previous investigation of tm mice highlighted the development of lupus-like disease marked by ANA deposition, GN, and hypergammaglobulinemia (13, 14, 24). Due to the suppressive atmosphere in tm24KO mice evidenced by increased MDSCs and Tregs, enhanced TGF-β axis, and decreased CD4 T cell activation, we wished to assess the manifestation of lupus-like disease in these mice as compared with tm mice. We first categorized ANA with a clinical score between 1 and 5, in which 5 is the strongest fluorescent intensity. Among n = 10 pairs of mice, ANA score was significantly increased in tm mice as compared with tm24KO mice. Representative panels for tm and tm24KO ANA results are shown (Fig. 5A). We next assessed GN in kidneys of tm or tm24KO mice. As categorized by fluorescent intensity (score 1–5), tm24KO mice showed decreased GN as compared with tm mice (n = 5 pairs) (Fig. 5B). Further, we performed H&E staining on kidneys from tm or tm24KO mice and counted nuclei per equal area section via 320 original magnification microscopy (13). The tm mice showed significantly more nuclei stain per field of area than tm24KO (Fig. 5C). These data indicate higher levels of endothelial and mesangial cells in glomeruli of tm mice. These data indicate significantly less autoimmune disease in tm24KO mice as compared with tm mice. Increased gammaglobulin levels are a hallmark of lupus-like disease. We measured serum levels of IgM, IgA, IgG1, IgG3, IgG2c, and IgG2b between tm and tm24KO mice (n = 5 pairs). Only IgM production was significantly less in tm24KO mice as compared with tm mice (Fig. 5C). Further analysis of B cell
activation and markers of plasma cell development suggested there were no detectable differences between B cell activity in tm and tm24KO mice (data not shown). These data suggest that B cells were not affected by the enhanced suppressive milieu or increased MDSC populations found in tm24KO mice. Taken together, these results showed a decreased progression of lupus-like disease in tm24KO mice in an immune-suppressive atmosphere marked by increased MDSCs, TGF-β axis, activated Tregs, and decreased naive T cell activation.

In the absence of CD24, free HMGB1 recruits MDSCs to impede progression of lupus-like disease

CD24 is a DAMP ligand for HMGB1 and HSPs (8). The role of soluble HMGB1 is not well defined, as studies show both a pro- and anti-inflammatory role for the cytokine (33). We wished to determine whether loss of CD24 led to altered levels of HMGB1 in the periphery of tm24KO mice. We performed Western blot on serum of tm and tm24KO mice and probed for HMGB1. We noted increased HMGB1 in WT and tm24KO sera as mice aged that correlated with progression of autoimmune disease. Along these lines, tm24KO mice showed greater levels of sera HMGB1 at 12 mo when compared with WT mice (Fig. 6A). Reports demonstrate that HMGB1 has the potential to recruit granulocytic and myeloid-derived cells (31, 34). To determine whether HMGB1 acted as a chemoattractant for myeloid and granulocytic populations in tm and tm24KO mice, we injected mice with low-dose (100 ng/mouse) HMGB1 and assessed myeloid cells in circulation prior to and 4 h postinjection. We found that in both tm and tm24KO mice, injection of HMGB1 caused significant increases in CD11b+Gr1+ populations found in blood 4 h postinjection (Fig. 6B). Unexpectedly, tm24KO mice showed significantly more recruitment of CD11b+Gr1+ cells as compared with tm mice. Data are plotted as percentage increase from baseline (Fig. 6C). Taken together, enhanced levels of HMGB1 in serum were likely due to loss of CD24, as levels were largely detectable in tm24KO, but not tm, mice. In the context of autoimmunity, free HMGB1 may act as a chemoattractant for MDSCs that leads to relative amelioration of lupus-like disease in tm24KO mice.

Discussion

CD24 has been shown to play important but apparently opposite roles in adaptive and innate immunity. On one hand, Chen et al. (8) demonstrated CD24 expressed on DCs binds to DAMPs and represses inflammatory responses by innate immune effectors. On the other hand, CD24 has been shown to be a costimulatory molecule on APCs for activation of CD4 and CD8 T cells to Ags (35, 36), especially in the context in which the host lacks robust costimulation by CD28 (36, 37). In addition, CD24 on T cells is essential for T cell homeostatic proliferation (38). In the context of autoimmune diseases, CD24 deficiency abrogates experimental autoimmune encephalomyelitis by preventing T cell expansion in the CNS (15) and promoting clonal deletion of autoreactive T cells (39, 40). The apparently opposite function of CD24 in inflammation and autoimmune diseases remain to be reconciled.
study, we investigated the role of CD24 in an autoimmune context in which endogenous DAMPs are consistently released.

Surprisingly, CD24 deficiency ameliorated clinical manifestations of autoimmune disease in a model of DAMP-driven autoimmune disease. Although CD24 deletion does not affect B cell activation and Ab production (Fig. 5), it reduces T cell activation, proliferation, and cytokine production (Fig. 2). We have previously shown that depletion of T cells leads to amelioration of the tm-driven autoimmune phenotype (14). The T cell proliferation defect that occurs in the absence of CD24 may play a role in decreased inflammatory T cell proliferation and activity in tm24KO mice, ultimately leading to decreased Th1-abetted autoimmunity.

We have reported enhanced suppressive capacity of Tregs in tm mice (24). In this study, we showed that Treg-suppressive capacity is further enhanced by CD24 deletion. Our data show an increase in absolute numbers of Tregs and greater IL-10 production in tm24KO mice. Correspondingly, we noted an increase in splenic IL-10 and TGF-β activity in tm24KO mice (Fig. 3). Moreover, perhaps because these cytokines are also associated with enhanced MDSC activation (10), we noted a significant increase in the MDSC population found in tm24KO mice. This population was heterogeneous in nature, consisting of Ly6C<sup>high</sup>/Gr1<sup>low</sup> and Ly6C<sup>low</sup>/Gr1<sup>high</sup> populations. Increased numbers of MDSCs in tm24KO may account for enhanced overall suppression of SLE-like symptoms (Fig. 5). MDSC expansion was consistent with increased TGF-β and diminished T cell activity in tm24KO mice (Figs. 2–4). Further, Ly6C<sup>high</sup>/Gr1<sup>low</sup> MDSCs from tm24KO mice have enhanced suppressive capacity as compared with the matched tm subset. Upon ligation with TLR4 ligand LPS, we saw increased nitrite release from Ly6C<sup>high</sup>/Gr1<sup>low</sup> MDSCs in tm24KO mice. These data point to a role for CD24 in regulation of NO<sub>2</sub> production and subsequent MDSC regulation. Taken together, the increased Treg and MDSC-suppressive activity in tm24KO mice is a causative factor in diminished effector T cell activation and SLE-like symptoms.

Nuclear protein HMGB1 is released from both necrotic and apoptotic cells and promotes inflammatory responses in both cancer and autoimmune diseases (41). The mechanisms through which HMGB1 promotes inflammation are not well defined, and it is important to note that cellular receptors for HMGB1 range from CD24 and TLRs to HSPs and CXCL12 (42). In autoimmune contexts, HMGB1 has been shown to exacerbate disease through several mechanisms. Nucleosomes containing HMGB1, detected in the serum of SLE patients, led to production of inflammatory cytokines IL-6, IL-1β, and TNF-α coupled to DC activation in a TLR2-dependent manner (43). SLE patients with inflammatory skin lesions release HMGB1 at the lesion site upon exposure to UVB (44). As a whole, reports show that HMGB1 is considered a potent inflammatory mediator in acute settings when associated with autoimmune disease. It is not understood how persistent low-level HMGB1 exposure will effect development of pro- and anti-inflammatory immunity in autoimmune diseases. The data presented in this study show that increased levels of HMGB1 appeared in tm24KO mice (Figs. 4. 6). Loss of CD24 as a potent HMGB1 receptor may explain increased free serum HMGB1 in tm24KO mice. We further show that, indeed, HMGB1 acts as a powerful chemoattractant for myeloid populations in both tm and tm24KO mice, and tm24KO mice recruited significantly more myeloid cells than tm mice in response to acute HMGB1 (Fig. 6). Thus, although CD24 also suppresses host response to HMGB1 in DAMP-driven autoimmune diseases, recruitment of MDSCs provided a new mechanism to explain the apparently opposite function of CD24 in adaptive and innate immunity.

Although the role for HMGB1 in MDSC development has not been studied, HMGB1 has been shown to promote regulatory cell activation that enables suppression of host antimurine immunity. For example, neutralization of tumor-derived HMGB1 blocks Treg-derived IL-10 and enables CD8<sup>T</sup> cells and IFN-γ production resultant in tumor rejection (45). Further, in head and neck cancers, it has been demonstrated that HMGB1 is a chemoattractant for Tregs and promotes their suppressive capabilities (46). Promotion of Treg-suppressive functions is associated with increased MDSC utility at the tumor site (47). In the context of cancer, it is well understood that suppressor cells are detrimental to host anti-tumor immunity. However, in inflammatory-mediated autoimmune diseases, Treg and MDSC populations may benefit the host. In keeping with this, cellular therapeutic strategies focus on restoration of immune tolerance through enhancement of these suppressor populations (48–50). The data presented in this study strongly support a potential role for Tregs and MDSCs in diminishing inflammation-mediated autoimmune manifestations. Further, the potential for low-level HMGB1 to drive these populations deserves further study.

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Disclosures

The authors have no financial conflicts of interest.

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DELETION OF CD24 REDUCES AUTOIMMUNITY THROUGH MDSC EXPANSION