



Disease Stage-Specific Pathogenicity of CD138 (Syndecan 1)-Expressing T Cells in Systemic Lupus Erythematosus

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CD138 (syndecan 1), a member of the heparan-sulfate proteoglycan family, regulates diverse biological responses by interacting with chemokines, cytokines, growth factors, and adhesion molecules. Expression of CD138 has been detected on T cells from both healthy and sick mice mimicking systemic lupus erythematosus (SLE) disease. However, the characteristics and the role of CD138+ T cells in SLE pathogenesis remain largely unknown. We analyzed the lupus-prone MRL/Lpr mice and the control MRL/MpJ strain as well as the common laboratory strains Balb/c, and C57BL/6 for CD138-expression and found that only the MRL/Lpr strain harbored TCRβ+CD138+ cells in various organs. The frequency of TCRβ+CD138+ cells progressively expanded in MRL/Lpr mice with age and correlated with disease severity. Majority of the TCRβ+CD138+ cells were CD4 and CD8 double-negative and 20% were CD4. At least a portion of TCRβ+CD138+ cells originated from CD4+ cells because substantial number of CD4+TCRβ+CD138- cells expressed CD138 after *in vitro* cultivation. Compared to TCRβ+CD138- cells, TCRβ+CD138+ cells exhibited central memory (Tcm) phenotype with reduced ability to proliferate and produce the cytokines IFNγ and IL-17. When co-cultured with B cells, the ability of TCRβ+CD138+ cells to promote plasma cell formation and autoreactive antibody production was dependent on the presence of autoantigen, CD4 co-receptor expression and cell-to-cell contact. Surprisingly, adoptively transferred TCRβ+CD138+ T cells slowed down disease progression in young recipient MRL/Lpr mice but had the opposite effect when DNA was co-administered with TCRβ+CD138+ T cells or when TCRβ+CD138+ cells were transferred to older MRL/Lpr mice with established disease. Thus, CD138-expressing T cells with Tcm phenotype enhance disease progression in SLE by rapidly activating autoreactive B cells when self-antigens are exposed to the immune system.

Keywords: lupus, syndecan-1, T cells, MRL/Lpr mouse, immunopathogenesis

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and inflammatory cell infiltration in multiple tissues (1). T cells are critical for SLE pathogenesis as they, not only provide help to autoreactive B cells, but also infiltrate and damage the target organs, such as the skin, joints, brain, lung, heart, and kidneys (2). T cells from SLE patients and lupus-prone mice have expanded T helper 1 (Th1), Th17, follicular, and extrafollicular helper T (T_{fh} and eT_{fh}) cell subsets, which contribute to the inflammation and autoreactive antibody production through increased IFN γ , TNF α , IL-6, IL-17, and IL-21 secretion (3–7). Diminished populations of T regulatory cells (Treg), T follicular regulatory (T_{fr}) cells, and cytotoxic CD8⁺ T cells were also thought to be contributing to the pathogenesis of SLE (8–11). In addition, increased circulating TCR $\alpha\beta$ +CD4-CD8- double negative T (dnT) cells and renal accumulation of the minor $\gamma\delta$ T population have been associated with autoantibody production and lupus nephritis (12). Besides the phenotypic and functional alterations in effector T cells, terminally differentiated memory T cells may also be contributing to the tissue damage as these cells accumulate in SLE patients with high disease activity (13–15).

Syndecans, type I transmembrane heparan sulfate proteoglycans (HSPG), regulate diverse biological processes, such as tissue wound repair, angiogenesis, epithelial-mesenchymal transformation, and inflammation, by modifying the local concentration, stability, and accessibility of extracellular matrix components, cytokines, chemokines, and growth factors (16). Syndecan family consists of four distinct members that are mostly expressed on epithelial, endothelial, neural, or fibroblastic cells, but they are also detected on haemopoietic cells (17, 18). Syndecan 2 and syndecan 4 are up-regulated upon CD4⁺ T cell activation and act as inhibitors by promoting T cell receptor (TCR) clearance or by activating tyrosine phosphatase CD148 (19, 20). Syndecan 1 (CD138) is commonly used as a marker to identify plasmablasts and plasma cells (21). Recently, CD138 has been suggested to be a marker to distinguish IL-17 producing natural killer T17 (NKT17) cells from other invariant NKT cells based on its selective expression on NKT17 cells but not on NKT1 and NKT2 cells (22, 23). Moreover, CD138⁺ T cells were observed accumulated in gut epithelia of aged C3H wild type as well as in the spleen and lymph nodes of FasL loss-of-function C3H gld mice (24). Similarly, CD138-expressing T cells were detected in spleen and lymph nodes of lupus prone μ MT/lpr mouse but these cells were only present in the lymph nodes, and not in the spleen, of another lupus-prone strain, B6/lpr mouse (25). Thus, accumulating evidence indicate the existence of CD138⁺ T cells in both healthy and diseased mice. However, the characteristics and pathologic roles of CD138⁺ T cells in lupus disease remain to be elucidated.

Here, we detected the presence of TCR β +CD138⁺ cells in various organs of the lupus-prone MRL/Lpr mice. The numbers of TCR β +CD138⁺ cells increased as the disease progressed. We also identified CD4⁺ T cells among the TCR β +CD138⁺ population as an important source of TCR β +CD138⁺ cells. These accumulating TCR β +CD138⁺ cells manifested mostly

central memory phenotype (T_{cm}) and promoted lupus disease progression only when autoantigens were present, despite exhibiting slower activation kinetics, reduced proliferation, and diminished cytokine production after stimulation with anti-CD3/CD28 antibodies.

MATERIALS AND METHODS

Mice

MRL/MpJ-*Fas*^{lpr}/J (referred to as MRL/Lpr throughout the manuscript), MRL/MpJ (referred to as MRL throughout the manuscript) mice, and C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Balb/c mice were purchased from Charles River Laboratories (Wilmington, MA). Only age-matched female mice were used for experiments. All mice were bred and maintained under specific pathogen-free conditions in the animal facility of US Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER) Veterinary Services. The breeding and use of animals were approved by the US FDA, CBER Institutional Animal Care and Use Committee (permit numbers 2002-37 and 2017-47).

Detection of Anti-dsDNA and SM Antibodies in Sera

Serum anti-dsDNA and anti-Smith antigen (SM) antibodies were measured by ELISA as described previously (26). Briefly, calf thymic DNA (Sigma-Aldrich, St. Louis, MO) or SM (Immunovision, Springdale, AR) were coated on 96-well microtiter plates (Dynatech Immulon 4 HBX; Dynatech Labs., Chantilly, VA) at 1 μ g/ml with 0.1 M of carbonate-bicarbonate buffer (pH 9.6) overnight at 4°C. Plates were blocked for 30 min at room temperature in 5% BSA in PBS, then washed with 0.05% Tween-20 in PBS. Diluted serum samples were added to wells in triplicates and incubated at 37°C for 2 h. Plates were washed with 0.05% Tween-20 in PBS and further incubated with HRP-conjugated goat antibodies directed against mouse IgG (Southern Biotech, Birmingham, AL) for 1 h at room temperature. Finally, plates were washed with 0.05% Tween-20 in PBS and measured at 405 nm absorbance after developing with ABTS solution (Invitrogen, Carlsbad, CA). Antibody titers were recorded as the last titration corresponding to the OD that is twice the mean OD of blank wells.

Flow Cytometry

Single cell suspensions of spleen, bone marrow, lymph nodes, and thymus were obtained by mechanic dissociation of tissue through a 40 μ m cell strainer. The dissociated cells were filtered through a 100 μ m cell strainer. Red blood cells were then lysed using ACK lysing buffer (Lonza, Wallersville, MD). In addition, mouse blood leukocytes were collected by lysing red blood cells with ACK lysing buffer and centrifugation at 300 \times g for 5 min. Cells were stained with fluorescent-conjugated anti-mouse antibodies after blocking CD16/CD32 with Fc Block (BD Biosciences, San Jose, CA). For intracellular staining, Brefeldin A (BD Biosciences)-treated cells were stained with the surface markers and LIVE/DEAD™ Fixable Near-IR Dead

cell kit (IR-Red) (Thermo Fisher, Waltham, MA) before fixation, permeabilization, and intracellular staining as per manufactures instructions (BD Biosciences). The following antibodies were used in flow cytometry analysis: Pacific blue anti-CD19, BV421 anti-CD19, BV421 anti-TCR β , APC anti-CD138, APC anti-TCR β , BV605 anti-CD3, FITC anti-CD3, Percp Cy5.5 anti-CD44, FITC anti-62L, PE-Cy7 anti-PD-1, APC anti-CXCR5, Percp Cy5.5 anti-B220, PE-Cy7 anti-CD8, PE anti-CD21, PE anti-CD22, BV421 anti-CD23, Alexa647 anti-CD40, FITC anti-CD80, FITC anti-CD86, Percp Cy5.5 anti-CD25, FITC anti-CD69, APC anti-CD95, Percp Cy5.5 anti-IL17, Percp Cy5.5 anti-CCR7, FITC anti-Foxp3 (all purchased from BioLegend, San Diego, CA). PE-anti-CD138 was purchased from BD Biosciences. In addition, FITC anti-BCMA, PE anti-TACI, FITC anti-IFN γ , Annexin V, (R&D system, Minneapolis, MN), ATTO 488 anti-BAFFR (Enzo life Science Inc., Farmingdale, NY), CellTrace™ CFSE Cell Proliferation Kit and Qdot605 anti-CD4 antibody (Thermo Fisher). Stained cells were acquired using LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo (Tree Star, Ashland, OR) version 10.1 for PC.

Quantitative Real-Time PCR

Total RNA was extracted from flow cytometry-sorted cells using the RNeasy Mini kit (Qiagen, Germantown, MD). Two hundred nanograms of total RNA were reverse-transcribed into cDNA using random hexamers with the Taqman Reverse transcription kit (Invitrogen). The expression of targeted genes and GAPDH were determined using Taqman Gene Expression assays and CFX96 Touch Real-Time System (BioRad, Hercules, CA). Relative expression values were determined by the $2^{-\Delta\Delta Ct}$ method where samples were normalized to GAPDH gene expression.

T Cell Isolation, Cultivation, and Adoptive Transfer Experiments

Splenic T cells from MRL/Lpr mice were purified with Dynabeads™ FlowComp™ Mouse Pan T (CD90.2) Kit and dissociated from beads as per manufacture's instructions (Thermo Fisher). Purified T cells were stained with PE-conjugated anti-CD138 antibody, and TCR β +CD138+ and TCR β +CD138- cells were further separated with anti-PE magnetic MicroBeads (Miltenyi Biotec, Auburn, CA). After three washes with PBS, the purity of isolated TCR β +CD138+ cells was >95% in all experiments as determined by flow cytometry. For *in vivo* transfer, purified TCR β +CD138+ and TCR β +CD138- cells were suspended in PBS and 1×10^7 cells in 100 μ l were i.v. injected into recipient mice. For *in vitro* culture, CD4+TCR β +CD138- cells were further isolated from purified TCR β +CD138- cells using the CD4 (L3T4) MicroBeads (Miltenyi Biotec), and unbound cells were identified as CD8+TCR β +CD138- cells (over 94% purity). To block mTOR, isolated CD4+TCR β +CD138- cells were cultured in the presence of 100 nM rapamycin (Tocris Biosciences, Minneapolis, MN). After 3 days of incubation cell viability as well as CD138 and CD4 expression levels were assessed in flow cytometry.

Co-culture of B Cells With T Cells

Splenic B cells were isolated from 5 or 12 weeks old MRL/Lpr mice using B Cell Isolation Kit (Miltenyi Biotec). The purity of isolated B cells was over 97%. B cells were stained with CFSE before co-culturing with purified TCR β +CD138+ or TCR β +CD138- cells in the presence of anti-CD3/CD28 antibodies (BD Biosciences), phorbol 12-myristate 13-acetate (PMA)/ionomycin, or autoantigens [1μ g/ml of DNA or SM (Immunovision)]. DNA was isolated from MRL/Lpr splenocytes by hyperthermo treatment at 42°C for 4 h. After 3 to 4 days of incubation, cells were analyzed for CFSE dilution by flow cytometry. In other assays, after 10 days of culture, culture supernatants were analyzed for antibody production as well as IL-2 and IFN γ secretion by ELISA (R&D Systems). In CD4 blocking experiments, Ultra-LEAF™ purified CD4 antibody (clone GK1.5, Biolegend), or control rat IgG (Sigma-Aldrich) were added to T and B cell co-cultures. In some co-culture experiments, T and B cells were incubated either as mixed or separated with 0.4 μ m pore sized polyester Corning Transwell® membrane insert (Sigma-Aldrich).

Pristane-Induced Lupus Model

Nine weeks old female Balb/c or C57BL/6 mice received a single i.p. injection of 0.5 ml pristane (Sigma-Aldrich) or 0.5 ml of sterile PBS. Fourteen weeks later, sera were collected and spleens were harvested. Sera were analyzed for auto-antibodies by ELISA, and splenocytes were subjected to flow cytometry for the presence of TCR β +CD138+ cells.

Evaluation of Disease Progression and Histopathological Assessment of the Kidneys

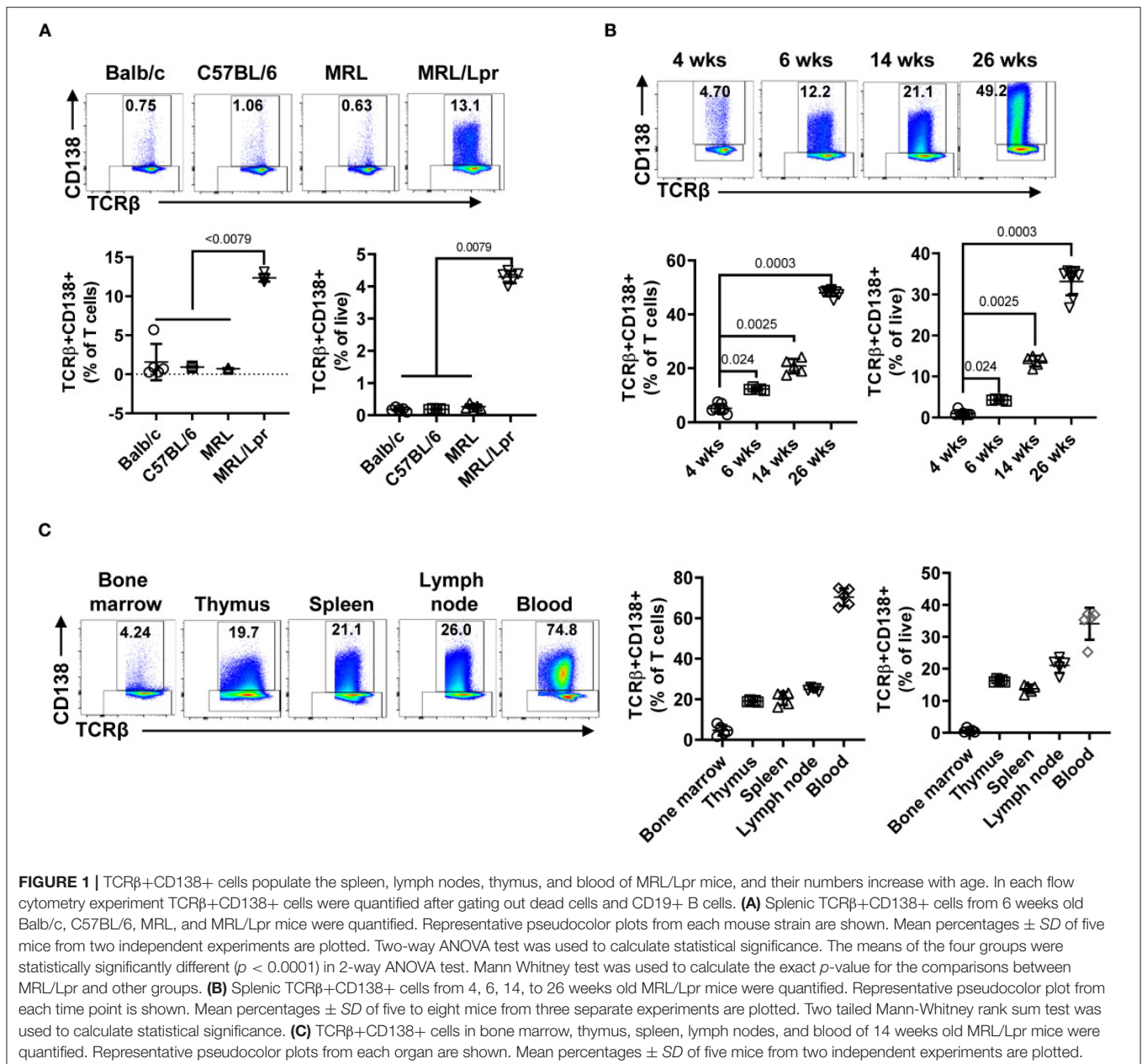
Proteinuria was measured using Fisherbrand™ Urine Reagent Strips (Fisher scientific, Hampton, NH) and scored on a scale of 0–5 (0, none; 1, trace; 2, 30 mg/dl; 3, 100 mg/dl; 4, 300 mg/dl; and 5, $\geq 2,000$ mg/dl).

Histopathological Analysis

Mouse kidneys were fixed in 10% buffered formalin overnight, processed, and embedded in paraffin. Sections were processed as previously described (26). Hematoxylin and eosin (H&E) and Masson trichrome stainings were performed. Stained slides were scanned by Nanozoomer XR (Hamamatsu corporation, Japan) and data was store as ndpi files for analysis. Overall severity, glomerular sclerosis, inflammatory cell accumulation, and interstitial fibrosis were evaluated and scored semiquantatively between 0 and 3 (0 = within normal limits, 1 = mild pathology, 2 = moderate pathology, and 3 = severe pathology). Average scores were analyzed by GraphPad Prism software (GraphPad, San Diego, CA).

Statistical Analysis

Data from groups were compared using GraphPad Prism software and non-parametric testing was performed by the Mann-Whitney rank sum two-tailed test for two groups and by two-way ANOVA on ranks for three or more groups.



Corresponding p -values for the test data points were listed on the figure.

RESULTS

Frequency of CD138-Expressing TCRβ+ Cells Increases Parallel to Disease Progression in MRL/Lpr Mice

The presence of CD138-expressing $\alpha\beta$ T cells has been reported in Fas and FasL mutant mouse strains (C3H gld, μ MT/lpr, and B6/lpr mice) manifesting lupus-like disease, but the physiological significance of CD138-expressing T cells in lupus

pathogenesis remains unexplored (24, 25). Here, we investigated the immunopathological role of CD138-expressing T cells in MRL/Lpr mouse, a widely used lupus-prone strain (27). First, we found a large fraction of CD138-expressing cells among CD19-TCRβ+ gated splenic population in MRL/Lpr mice (6 weeks old), even before the onset of autoimmune manifestations (Figure 1A, Supplemental Figure 1A). Measurement of CD138 mRNA in qRT-PCR assay on sorted TCRβ+CD138+ cells confirmed the expression of CD138 (Supplemental Figure 1B). However, the frequency of TCRβ+CD138+ cells in the spleens of age-matched Balb/c, C57BL/6 as well as the parental MRL mice remained negligible (Figure 1A). Thus, the expression of CD138 on T cells appears to be uniquely associated with Fas

signaling deficiency (24, 25). Second, we investigated a possible correlation between TCR β +CD138+ cells and the progression of disease in MRL/Lpr mice and found that the frequency of TCR β +CD138+ cells gradually increased with the age of mice (Figure 1B). Moreover, the increase in TCR β +CD138+ cell population was also detected in pristine-injected Balb/c and C57BL/6 mice (Supplemental Figures 1C,D), which also develop lupus-like autoimmune symptoms (27). It is important to note that although TCR β +CD138+ cells were previously reported to be confined to the lymph nodes in B6/lpr mice (25), we found high percentage of these cells also in the thymus, spleen, lymph nodes, and blood of MRL/Lpr mice (Figure 1C). As in MRL/Lpr mice, higher frequencies of TCR β +CD138+ cells were also found in the thymus, lymph node and blood of pristane-treated Balb/c mice (Supplemental Figure 1E). The increase in the frequency of blood TCR β +CD138+ cells in 14-week old MRL/Lpr and pristane-treated mice suggest extensive circulation of TCR β +CD138+ cells in lupus mice when they develop severe lupus disease.

TCR β +CD138+ Cells Derive From CD138-CD4+ T Cells

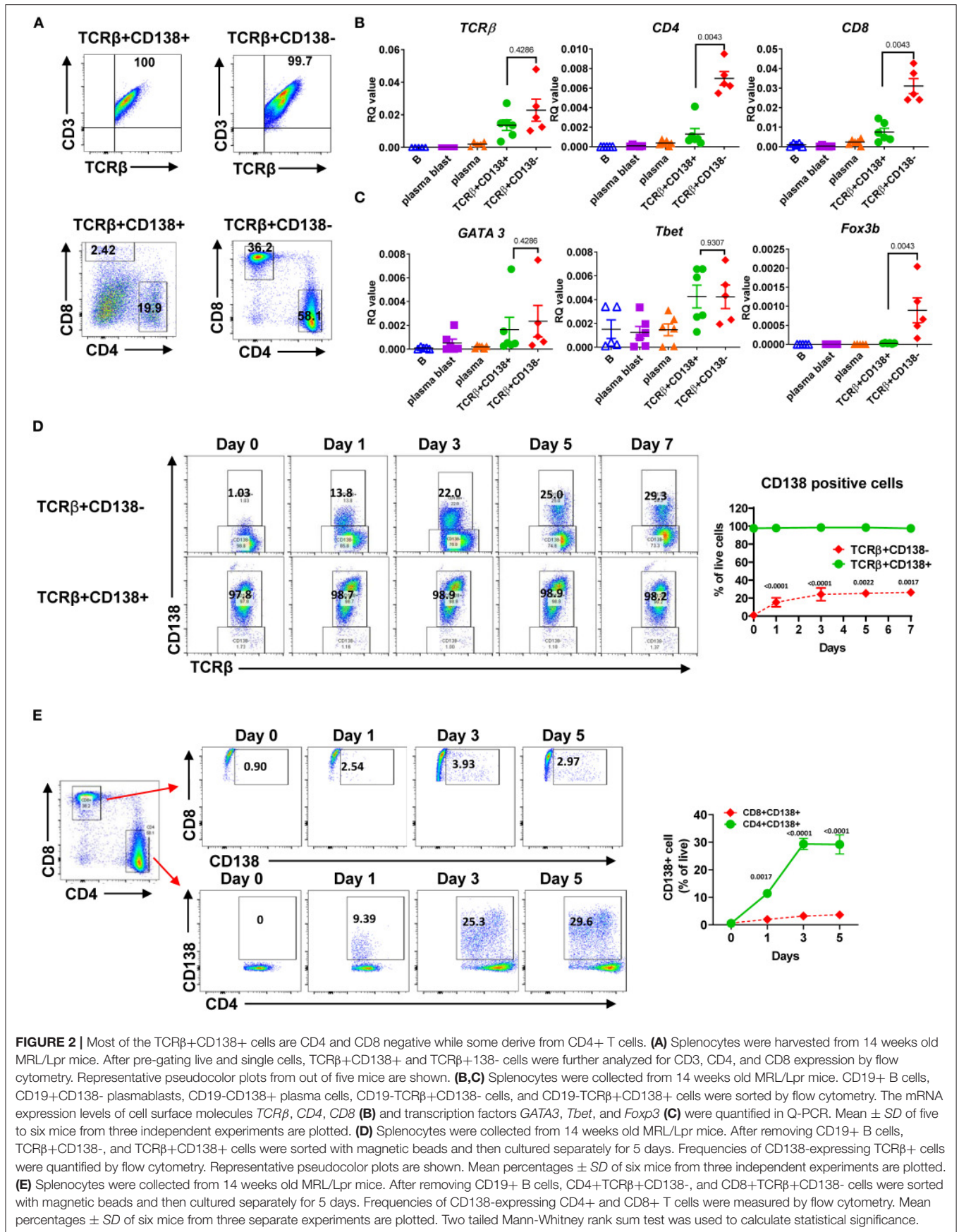
Next, we further characterized the MRL/Lpr mice TCR β +CD138+ cells by assessing the expression of surface markers and the genes associated with B and T cell lineages. As shown previously in μ MT/lpr and B6/lpr mice (24, 25), most of the TCR β +CD138+ cells and only about 5% of TCR β +CD138- cells in MRL/Lpr mice expressed B220 (Supplemental Figure 2A). There was no apparent difference in the expression of other B cell-related surface proteins BAFFR, BCMA, IgM, CD21, CD23, CD40, CD80, or CD86 as well as mRNA for *CD19*, *IGHM*, *Myc*, *tnfrsf13c* (BAFFR), and *tnfrsf13b* (TACI) between TCR β +CD138+ and TCR β +CD138- cells (Supplemental Figures 2B–D). In addition, the expression levels of transcription factors (*Pax5*, *Pu1*, *Irf4*, and *Xbp1*) associated with B and plasma cell-differentiation were comparable between TCR β +CD138+ and TCR β +CD138- cells, although *Bcl-6* was higher and *Prdm1* was lower in CD138-expressing cells (Supplemental Figure 2E). CD138 expression did not affect the expression of the T cell marker CD3 on TCR β + cells as both TCR β +CD138+ and TCR β +CD138- were CD3+ (Figures 2A,B). In μ MT/lpr and B6/lpr mice, all TCR β +CD138+ cells were reported to be negative for CD4 and CD8 (24, 25). Although the majority of TCR β +CD138+ cells were negative for CD4 and CD8 in MRL/Lpr mice also, ~20% of TCR β +CD138+ were CD4+, while only ~2% were CD8+ (Figures 2A,B). Further confirming the association of TCR β +CD138+ cells with T-cell but not with B-cell lineage, we measured comparable levels of *GATA3* and *Tbet* expression in TCR β +CD138+ and TCR β +CD138- cells, the transcription factors associated with Th2 and Th1 subsets (28), respectively (Figure 2C). Interestingly, CD138- cells expressed the Treg cell transcription factor *Foxp3* but not CD138-expressing cells (Figure 2C). To assess whether CD138 expression changed over time in incubated TCR β + T cells, we assessed the changes in the percentage of CD138+ cells

in cultured TCR β +CD138+ and TCR β +CD138- cells *in vitro*. As shown in Figure 2D, CD138 levels remained high on TCR β +CD138+ cells throughout the 7-days culture period. By contrast, there was a gradual increase in the frequency of CD138+ cells among the cultured TCR β +CD138- cells from day 1 to 3. We next sought to determine whether CD138+ cells emerged from CD4+TCR β +CD138- or CD8+TCR β +CD138- subsets. Incubation of highly purified CD4+TCR β +CD138- and CD8+TCR β +CD138- for 5 days indicated that a substantial number of CD138+ cells derived from CD4+ cells. As shown in Figure 2E and paralleling the kinetics of CD138 expression among the TCR β +CD138- population in Figure 2D, a significant increase in CD138 expression was observed among the CD4+ cells on day 1 which plateaued on day 3, while the increase in CD138+ population among the CD8+ cells remained limited. These results suggested that a portion of CD4+TCR β +CD138- cells expresses CD138 after culture.

Multiple transduction pathways, including (mammalian target of rapamycin) (mTOR), phosphatidylinositol-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK), control T cell survival, metabolism, and differentiation (29). Besides, elevated mTOR activity was found to control the expansion of dnT cells (30–32). Moreover, mTOR promotes the differentiation of CD138-expressing plasma cells (33). To test whether these pathways were also critical for CD138 expression on lupus T cells, we treated CD4+TCR β +CD138- cells with rapamycin, a specific mTOR inhibitor, and assessed the increase in CD138 expression. As reported previously (34), rapamycin significantly reduced cell survival compared to media or DMSO treated cells after 3 days of incubation (Supplemental Figure 2F). As observed in Figure 2E, around 30% of the CD4+TCR β +CD138- cells incubated in media expressed CD138 after 3 days of incubation (Supplemental Figure 2G). A similar increase in CD138 expression was observed in cells incubated in DMSO. Although, rapamycin treatment did not reduce the CD4 expression on the cell surface, as suspected, the inhibition of mTOR pathway significantly decreased both the CD138 expression level and the frequency of CD138+ cells (Supplemental Figure 2G). Thus, mTOR signaling pathway may be involved in the expression of CD138 on CD4+TCR β +CD138- cells.

TCR β +CD138+ Cells Respond Less to TCR Engagement and PMA/ionomycin Activation Compared to CD138-Negative Counterparts

After TCR activation, T cells undergo a series of events, including up-regulation of cell surface markers, proliferation, apoptosis, and cytokine secretion (35). We assessed the differences in proliferation of TCR β +CD138+ and TCR β +CD138- cells following stimulation with anti-CD3/CD28 antibodies. Compared to TCR β +CD138- cells, TCR β +CD138+ cells proliferated significantly less at 48- and 72-h time points (Figure 3A). Stimulated cells were also assessed for apoptosis by flow cytometry. Compared to TCR β +CD138- cells, TCR β +CD138+ cells had higher number of live cells accompanied by lower number of early apoptotic and necrotic



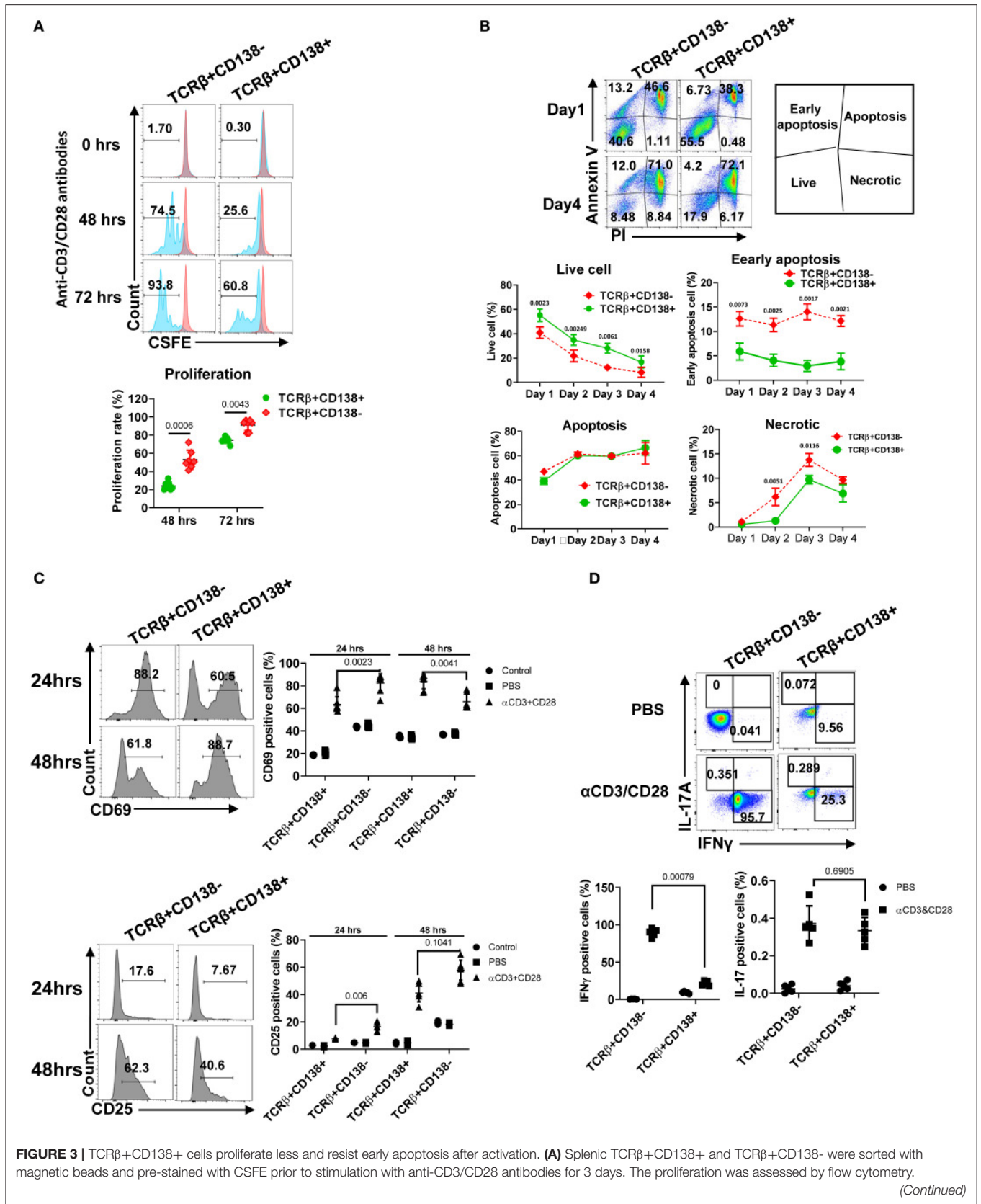


FIGURE 3 | Representative histogram images are shown. Mean percentages \pm SD of seven mice from three separate experiments are plotted. **(B)** Sorted splenic TCR β +CD138+ and TCR β +CD138- were stimulated for 4 days with anti-CD3/CD28 antibodies. Live, early apoptotic, apoptotic, and necrotic cells were measured by flow cytometry. Representative pseudocolor plots are shown. Mean percentages \pm SD of six mice from three independent experiments are plotted. **(C)** Sorted splenic TCR β +CD138+ and TCR β +CD138- cells were activated with anti-CD3/CD28 antibodies for 24 or 48 h. Cells were stained with CD69 and CD25 antibodies to assess activation kinetics in flow cytometry. Representative histogram images indicating the frequency of positive cells are shown. Mean percentages \pm SD of seven mice from three independent experiments are plotted. **(D)** Sorted splenic TCR β +CD138+ and TCR β +CD138- cells were activated with anti-CD3/CD28 antibodies for 16 h. Representative pseudocolor plots show intracellular IFN γ , and IL-17 staining. For each cytokine, mean \pm SD of five mice are plotted. Two tailed Mann-Whitney rank sum test was used to calculate statistical significance.

cells (**Figure 3B**). The early and late activation state of TCR-stimulated T cells were assessed by CD69 and CD25 expression, respectively (36). Overall, anti-CD3/CD28 antibody-induced activation of TCR β +CD138+ cells were delayed compared to TCR β +CD138- cells. At 24-h time point, CD69 expression was higher on TCR β +CD138- cells than on TCR β +CD138+ cells, but by 48 h its expression decreased compared to TCR β +CD138+ cells (**Figure 3C**). Conversely, the increase in CD69 expression on TCR β +CD138+ cells peaked with a delay at 48 h. A similar delay in the activation markers were observed in PMA/ionomycin-stimulated TCR β +CD138+ cells (**Supplemental Figure 3A**). In SLE, activated T cells participate in the inflammatory process through the production of cytokines such as IFN γ , TNF α , and IL-17 (2–4, 7, 37, 38). We found that after TCR engagement and PMA/ionomycin stimulation more than 90% of TCR β +CD138- cells were positive for IFN γ , while <30% of TCR β +CD138+ cells produced IFN γ (**Figure 3D**, **Supplemental Figures 3B,F**). Similarly, TCR β +CD138+ cells expressed less TNF α and IL-21 compared to TCR β +CD138- cells (**Supplemental Figures 3C,D**). Unlike IFN γ and TNF α , IL-17 production was not different between the two subsets after TCR stimulation (**Figure 3D**, **Supplemental Figure 3E**). However, TCR β +CD138+ cells produced less IL-17 than TCR β +CD138- cells after PMA/ionomycin stimulation (**Supplemental Figure 3F**). These results indicate that the phenotype of TCR β +CD138+ cells activated with either PMA/ionomycin or through TCR are markedly different than their CD138 deficient counterparts.

TCR-Stimulated TCR β +CD138+ Cells Are Less Efficient in Activating B Cells Than TCR β +CD138- Cells

An important function of T cells is to enhance antibody-mediated immunity by driving B cell proliferation and development into long lived memory B cells or antibody-secreting plasma cells (39). To investigate the capacity of TCR β +CD138+ in helping B cells, we co-incubated splenic B cells purified from 6 weeks old (disease free) MRL/Lpr mice with splenic TCR β +CD138+ or TCR β +CD138- cells from 10 to 12 weeks old MRL/Lpr mice in the presence of anti-CD3/CD28 antibodies and determined the proliferation of B cells (**Supplemental Figure 4A**). We found significantly less proliferation of B cells co-cultured with TCR β +CD138+ cells than those co-cultured with TCR β +CD138- cells (**Figure 4A**). Although TCR β +CD138+ cells harbor higher *Bcl-6* (**Supplemental Figure 2E**), a transcription factor for CXCR5+PD-1+ T follicular helper (Tfh)

cells, than TCR β +CD138- cells, in B cell co-culture experiments the increase in Tfh cell numbers among TCR β +CD138+ cells was modest and remained less than the increase in TCR β +CD138- cells (**Supplemental Figures 4A,B**). Moreover, TCR β +CD138+ cells were less efficient in inducing the generation of plasma cells (**Figure 4B**) and the production of IgM and IgG than TCR β +CD138- cells (**Figure 4C**).

To further characterize TCR β +CD138+ cells, we tested the immunomodulatory effect of TCR β +CD138+ cells in SLE by adoptively transferring TCR β +CD138+ or TCR β +CD138- cells into MRL/Lpr mice and evaluating the disease progression. We conducted the adoptive transfer experiments in two different recipient mice age groups, 7 to 8 weeks old mice with minimal lupus symptoms and 11 to 12 weeks old mice with established lupus symptoms. We chose these two age groups to assess whether the CD138-expressing cells impact the lupus progression differently in MRL/Lpr mice from different stages of disease. To our surprise, TCR β +CD138+ cells had completely opposite effect in the recipient mice at different ages. The 7 to 8 weeks old recipient mice without lupus symptoms manifested slower progression of disease when they were transferred with TCR β +CD138+ cells from 10 to 12 weeks old MRL/Lpr (sick) mice, compared to those that were injected with PBS. The increase in anti-dsDNA antibody and proteinuria levels were slower compared to those that were injected with PBS (**Figure 4D**, **Supplemental Figure 4C**). Kidney histopathological findings showed the most severe changes in recipients of TCR β +CD138- group, including end stage glomerulosclerosis with severe inflammation and interstitial fibrosis. The glomerular histopathological changes of TCR β +CD138+ cell-recipient mice were reduced as compared to PBS-injected mice 2 weeks after the transfer of cells, but the difference in histopathological scores did not reach statistical significance (**Figure 4E**). However, histopathological changes were significantly less in TCR β +CD138+ cell-injected mice than those injected with TCR β +CD138- cells. In sharp contrast to its effect in young recipient mice, TCR β +CD138+ cells significantly increased the development of anti-dsDNA antibodies and proteinuria in older MRL/Lpr mice with existing disease symptoms (**Figures 4F**, **Supplemental Figure 4D**). The TCR β +CD138- cells accelerated the disease progression in recipient animals from both age groups but their impact on older recipient mice was not as pronounced as those that received TCR β +CD138+ cells (**Figures 4D–F**, **Supplemental Figures 4C,D**). Together with the *in vitro* B cell co-incubation data (**Figures 4A–C**), the adoptive transfer experiments in young MRL/Lpr mice suggest an immunosuppressive function for TCR β +CD138+ cells on B

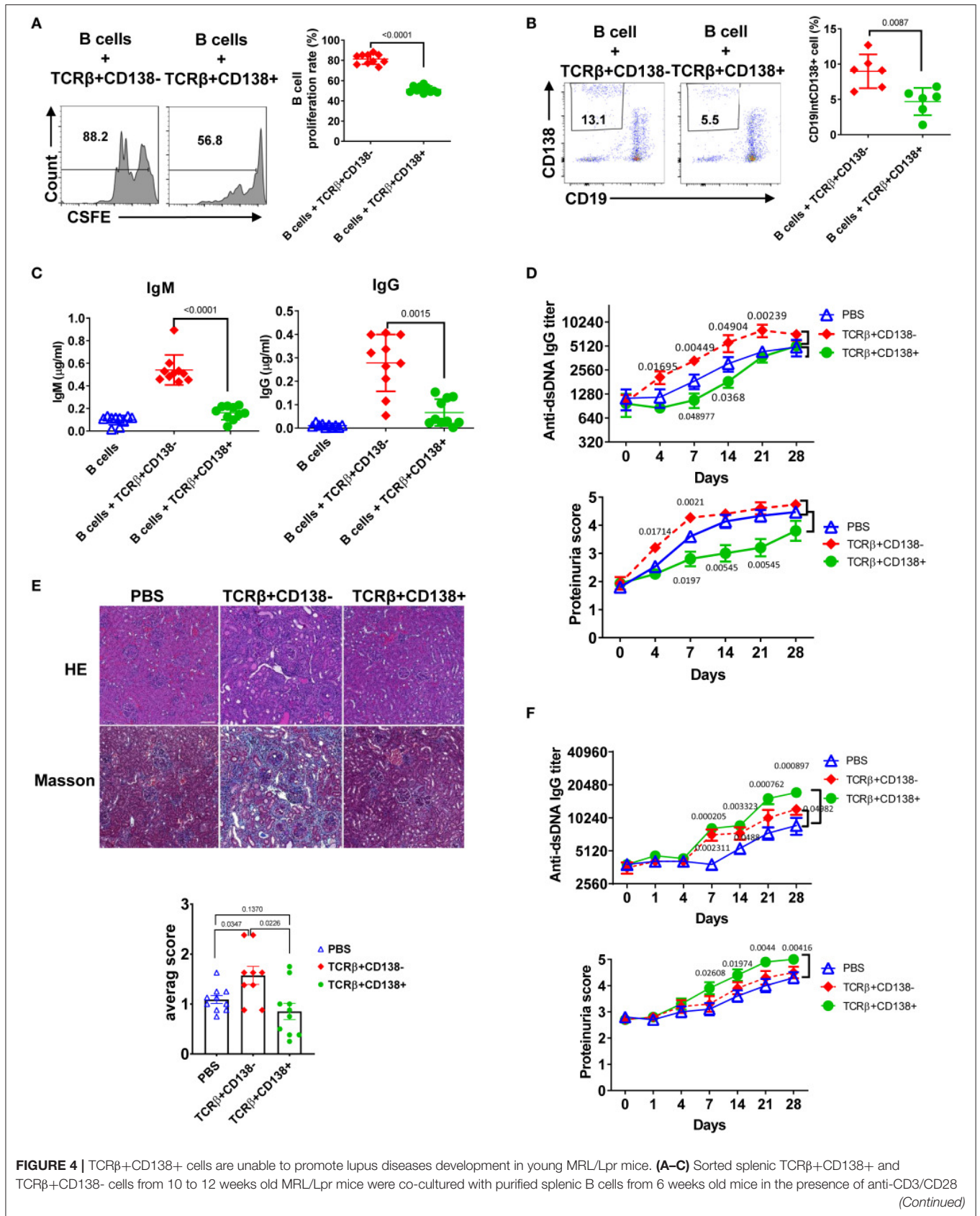


FIGURE 4 | TCRβ+CD138+ cells are unable to promote lupus diseases development in young MRL/Lpr mice. (A–C) Sorted splenic TCRβ+CD138+ and TCRβ+CD138- cells from 10 to 12 weeks old MRL/Lpr mice were co-cultured with purified splenic B cells from 6 weeks old mice in the presence of anti-CD3/CD28 (Continued)

FIGURE 4 | antibodies for 5 days. After gating-out TCR β + T cells, the proliferation of B cells **(A)** and the frequency of plasma cells (CD19^{int}CD138+) **(B)** were measured by flow cytometry. Mean \pm SD of 10 mice **(A)** or six mice **(B)** from three independent experiments are plotted. **(C)** Culture supernatants from the above co-culture experiment were analyzed for total IgM and IgG concentrations by ELISA. Mean \pm SD of 10 mice from three independent experiments are plotted. **(D,E)** Splenic TCR β +CD138+ and TCR β +CD138- cells were sorted from 10 to 12 weeks old MRL/Lpr mice and then adoptively transferred into 7 to 8 weeks old MRL/Lpr mice without disease symptoms. **(D)** Autoreactive IgG antibody (dsDNA) and proteinuria levels were measured on indicated days. Mean \pm SEM of 15 mice from three independent experiments are plotted. **(E)** Kidneys were collected 2 weeks after the transfer of cells and histopathological evaluations were performed on H&E and Masson stained specimens. Average pathology scores of 10 mice from two separate experiments are plotted. **(F)** Splenic TCR β +CD138+ and TCR β +CD138- cells were sorted from 10 to 12 weeks old MRL/Lpr mice and then adoptively transferred into 11 to 12 weeks old MRL/Lpr mice with existing disease symptoms. Autoreactive IgG antibody (dsDNA) and proteinuria levels were measured on indicated days. Mean \pm SEM of 10 mice from two independent experiments are plotted. Two tailed Mann-Whitney rank sum test was used to calculate statistical significance.

cell differentiation. However, TCR β +CD138+ cells contribute to the acceleration of disease progression if the recipient host has established lupus disease.

TCR β +CD138+ Cells Augment Autoreactive B Cell Responses When Auto-Antigens Are Present

Next, we sought to explore the underlying mechanism for the dramatic difference in the disease-stage-specific impact of TCR β +CD138+ cells to lupus progression in the adoptive transfer experiments. An important distinction between the young and older recipient MRL/Lpr mice in the adoptive transfer experiments is the exposure of the immune system of older mice (11–12 weeks old), but not the younger (7–8 weeks old) mice, to self-antigens as a result of apoptosis (40). As observed with the younger mice in the adoptive transfer experiments, *in vitro* TCR stimulation of TCR β +CD138+ cells also resulted in less activation of autoreactive B cells than those culture conditions containing TCR-stimulated TCR β +CD138- cells. A common feature of these *in vitro* and *in vivo* experiments is the absence of auto-antigens in the system. Earlier studies have highlighted the importance of B cells in presenting auto-antigens to T cells in activating autoreactive T cells (41). We therefore repeated the *in vitro* co-culture experiments in the presence of apoptotic DNA instead of anti-CD3/CD28 antibodies. Interestingly, when B cells from 12 weeks old MRL/Lpr mice with established disease were co-incubated with TCR β +CD138+ cells from age matched MRL/Lpr mice in the presence of DNA from apoptotic cells, culture supernatants contained significantly higher autoreactive **(Figure 5A)** and total IgG and IgM antibodies than those co-cultured with TCR β +CD138- cells **(Supplemental Figure 5A)**. Inclusion of DNA in the co-culture experiments resulted in a higher percentage of Tfh cell formation from TCR β +CD138+ cells than when cells were stimulated with anti-CD3/CD28 antibodies **(Supplemental Figure 4B vs. Supplemental Figure 5B)**. Despite this increase, the frequencies of Tfh cells among TCR β +CD138- and TCR β +CD138+ cells were comparable **(Supplemental Figure 5B)**. Nevertheless, consistent with the difference in antibody responses, higher percentage of plasma cells (CD3-CD19-CD138+) emerged from the cultures containing TCR β +CD138+ cells than from the cultures with TCR β +CD138- cells **(Figure 5B)**. Increased plasma cell generation and autoreactive antibody production with TCR β +CD138+ cells were not restricted to self-DNA-containing cultures, because replacement of self-DNA with the

auto-antigen SM also resulted in higher percentage of plasma cell development and increased production of anti-SM antibodies with TCR β +CD138+ cells than with TCR β +CD138- cells **(Figure 5C, Supplemental Figure 5C)**. Thus, regardless of the nature of the autoantigen, TCR β +CD138+ cells are more potent in aiding autoreactive B cells to produce self-reactive antibodies than TCR β +CD138- cells.

We next sought to determine whether TCR β +CD138+ cell-enhanced B cell differentiation is mediated by direct cell contact, especially because the majority of the MRL/Lpr mice TCR β +CD138+ cells were CD4 and CD8 negative **(Figure 2A)**. Separation of B and TCR β +CD138+ cells in the co-culture experiments with a Transwell[®] system resulted in a significant reduction in plasma cell generation compared to cells cultured without a Transwell[®] system **(Figure 5B)**. Moreover, although CD4+ cells constituted only ~20% of the TCR β +CD138+ cells, the B cell help provided by TCR β +CD138+ cells required CD4 because inclusion of anti-CD4 blocking antibodies in the co-culture system severely reduced plasma cell development and production of anti-dsDNA IgG and IgM antibodies **(Figure 5D, Supplemental Figure 5D)**. These experiments established the CD4+TCR β +CD138+ cells as more potent autoreactive B cell-activating T cell subset when self-antigens are present in the culture environment.

TCR β +CD138+ Cells Promote Disease in MRL/Lpr Mice Only When Self-Antigens Are Exposed

We showed that in order for TCR β +CD138+ cells to augment autoreactive B cell responses, they need to be stimulated by B cell-presented self-antigens **(Figures 5A–D)**. The fact that the recipient MRL/Lpr mice used in the adoptive transfer experiments in **Figure 4D** were too young to have sufficient amounts of circulating self-antigens, such as DNA, could be the reason why the transferred TCR β +CD138+ cells were not activated and did not exacerbate SLE symptoms in the recipient mice. Conversely, the acceleration of disease progression in older MRL/Lpr mice after the transfer of TCR β +CD138+ cells could be due to the presence of circulating self-antigens. To test this possibility, we co-administered TCR β +CD138+ cells with DNA into young (5 to 6 weeks old) MRL/Lpr mice. As observed previously **(Figure 4D)**, the increase in anti-dsDNA IgG and IgM antibodies as well as proteinuria were significantly slower in mice injected only with TCR β +CD138+ cells compared to PBS-injected mice or control mice injected with DNA only

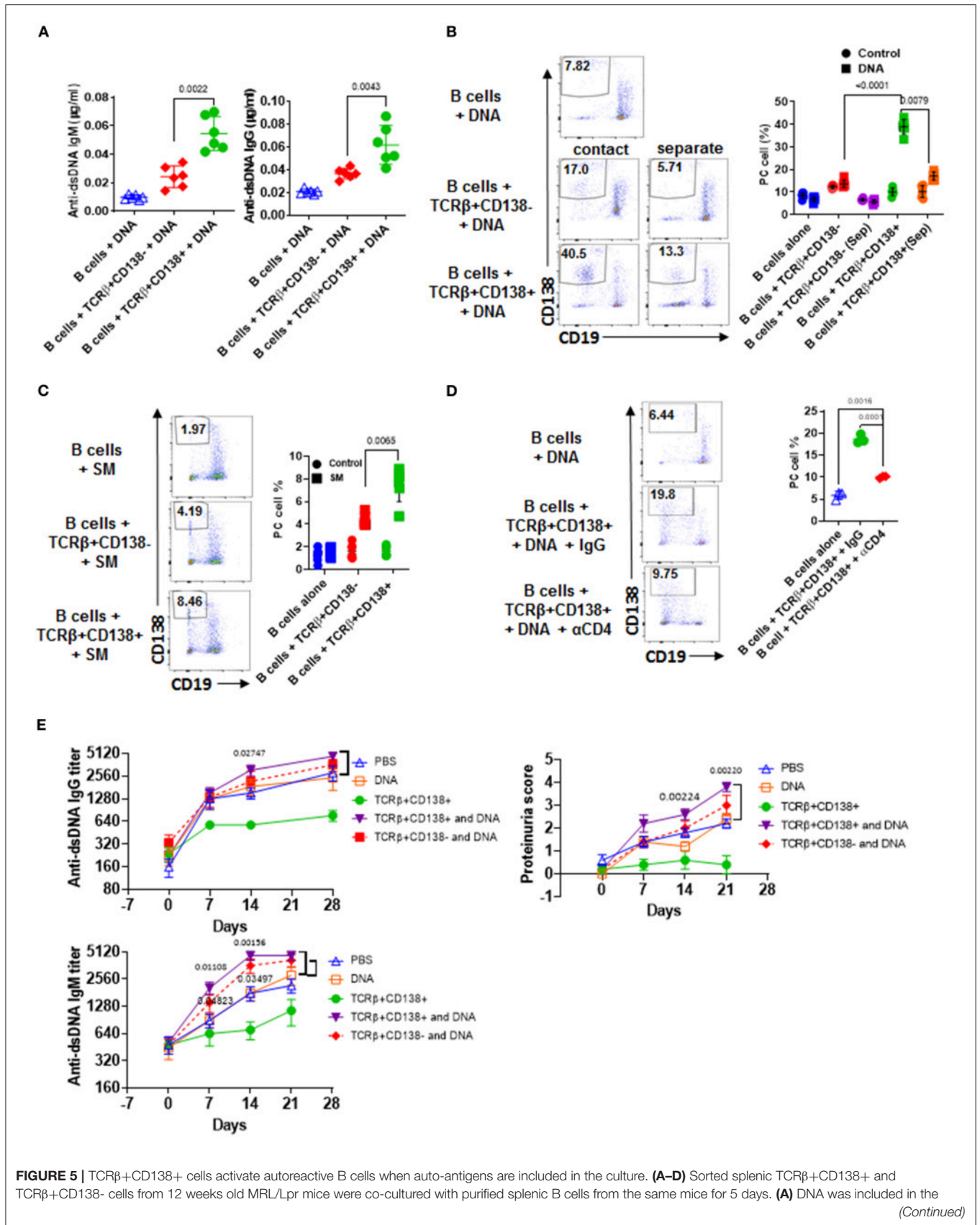


FIGURE 5 | TCRβ+CD138+ cells activate autoreactive B cells when auto-antigens are included in the culture. **(A–D)** Sorted splenic TCRβ+CD138+ and TCRβ+CD138- cells from 12 weeks old MRL/Lpr mice were co-cultured with purified splenic B cells from the same mice for 5 days. **(A)** DNA was included in the (Continued)

FIGURE 5 | co-cultured cells and culture supernatant anti-dsDNA IgM and IgG antibodies were measured by ELISA. Mean \pm SD of six mice from three independent experiments are plotted. **(B)** T and B cells were incubated as mixed (contact) or separated with Transwell® (separate) in the presence of DNA. After gating-out TCR β + T cells, the differentiation of B cells into plasma cells (CD19^{int}CD138+) was quantified by flow cytometry. Mean percentages \pm SD of six mice from three independent experiments are plotted. **(C)** SM was included in the co-cultured cells and differentiation of B cells into plasma cells (CD19^{int}CD138+) were quantified by flow cytometry after gating-out TCR β + T cells. Mean percentages \pm SD of six mice from three experiments are plotted. **(D)** Cells were incubated in the presence of antibody against CD4 or control IgG and the differentiation of B cells into plasma cells was quantified by flow cytometry. Mean percentages \pm SD of three independent experiments are plotted. **(E)** Splenic TCR β +CD138+ and TCR β +CD138- cells from 10 to 12 weeks old MRL/Lpr mice were sorted and then adoptively transferred into 5 to 6 weeks old MRL/Lpr mice with or without DNA. Mice that received PBS or DNA only served as control. Serum anti-dsDNA IgG and IgM antibody as well as proteinuria levels at indicated days were measured. Mean \pm SEM of five mice are plotted. Two tailed Mann-Whitney rank sum test was used to calculate statistical significance.

(Figure 5E). In contrast, and as hypothesized, anti-dsDNA antibody, and proteinuria levels were significantly higher in mice co-administered with DNA and TCR β +CD138+ cells than in control mice injected with DNA only (Figure 5E). Taken together, TCR β +CD138+ cells can modulate lupus development in MRL/Lpr mice in a disease-stage-dependent manner; they slow down the symptoms prior to the emergence of self-antigens and accelerate the disease progression when self-antigens are exposed.

TCR β +CD138+ Cells Are Central Memory Biased T Cells

Abnormal accumulation and differentiation of memory T cells have been reported in lupus patients (13). Memory T cells confer immediate protection and mount recall responses upon reencounter with antigens. Since adoptively transferred TCR β +CD138+ cells promoted disease progression in an autoantigen dependent manner (Figure 5), we asked whether TCR β +CD138+ cells have a memory T cell phenotype. The circulating memory T cell compartments are divided into Tcm and effector memory T cells (Tem) subsets based on the expression of cell surface molecules, such as CD44, CD62L, and CCR7 (42). We first characterized the expression of CD44 and CD62L on splenic TCR β +CD138+ and TCR β +CD138- cells in 12 weeks old MRL/Lpr mice. Using these two markers, we identified CD44-CD62L+ naïve T (Tn) cells, CD44+CD62L- Tem, and CD44+CD62L+ Tcm subsets. Among the TCR β +CD138- cells, ~10% were Tn, 50% Tem, and 40% Tcm (Figure 6A, Supplemental Figure 6A). Interestingly, Tcm cells comprised the vast majority (95%) of the population among the TCR β +CD138+ cells (Figure 6A, Supplemental Figure 6A). Typically, memory T cells are either CD4+ or CD8+ (43), but in MRL/Lpr mice, the majority of the CD138-expressing CD4+, CD8+ as well as CD4-CD8- cells exhibited Tcm memory phenotype, based on the elevated expression of CD44 and CD62L (Supplemental Figure 6B).

The chemokine receptor CCR7, which enables cells to home to secondary lymphoid organs where they encounter antigen, are highly expressed on Tcm cells (44). Further confirming their Tcm phenotype, TCR β +CD138+ cells expressed higher CCR7 levels than TCR β +CD138- cells (Figure 6B). The Tcm and Tem memory subsets can also be distinguished based on the expression of transcription factors *Bcl-6* and *Bim* for Tcm and BLIMP1 (*Prdm1*) for Tem, respectively (45, 46). We found higher *Bcl-6* and *Bim1* but lower *Prdm1* mRNA expression in TCR β +CD138+ cells, compared to TCR β +CD138- cells

(Figure 6C). We also assessed Tcm phenotype based on IFN γ and IL-2 production because in humans, Tcm cells produce more IL-2, while Tem cells are distinguished by high IFN γ and TNF α production (47). Consistent with data in humans upon stimulation with anti-CD3/CD28 antibodies, MRL/Lpr mice TCR β +CD138+ cells secreted significantly higher IL-2 but lower IFN γ than TCR β +CD138- cells (Figure 6D). The Tcm specific IL-2 and IFN γ production profile in TCR β +CD138+ cells were also observed in co-culture system with autoantigens and B cells (Figure 6E). Moreover, we found that in MRL/Lpr mice, the Tcm phenotype of TCR β +CD138+ cells were not restricted to the spleen or the age because cells isolated from various organs of mice at different ages, all predominantly exhibited the Tcm phenotype (Figure 6F, Supplemental Figures 6B,C). Collectively, these data established the memory phenotype of TCR β +CD138+ cells as Tcm in MRL/Lpr mice. Finally, to assess whether the Tcm phenotype of TCR β +CD138+ is unique to MRL/Lpr mouse, we compared the memory phenotype of TCR β +CD138+ cells from Balb/c and C57BL/6 mice to those of MRL/Lpr mice. Again, regardless of the mouse strain, TCR β +CD138+ were CD44+CD62L+ Tcm cells (Figure 6G). Thus, the Tcm phenotype of TCR β +CD138+ cells is conserved among different mouse strains.

DISCUSSION

In this study, we uncovered a disease-stage dependent accumulation of TCR β +CD138+ cells in various organs of lupus-prone MRL/Lpr mice, which are overwhelmingly positive for B220, but negative for CD4 and CD8 expression. Although these cells are less efficient in responding to non-specific T cell stimuli, such as PMA/ionomycin and anti-CD3/CD28 antibody engagement, they are more potent in aiding antibody production from autoreactive B cells *in vitro* as well as *in vivo* when autoantigens are present. Further characterization established the phenotype of these cells as Tcm based on high expression levels of CD62L, CD44, CCR7, and Bcl-6.

CD138 is widely expressed on epithelial cells as well as on other adherent cells, but its expression on normal lymphoid cells has been thought to be restricted to plasma cells and pre-B cells. However, recent studies reported that CD138 is also present on NKT17 and GC B cells, where it may be involved in host defense or autoimmunity through IL-17 secretion or binding to death receptor 6 on Tfh cells (22, 48). In aged C3H mice, accumulation of CD138-expressing T cells was

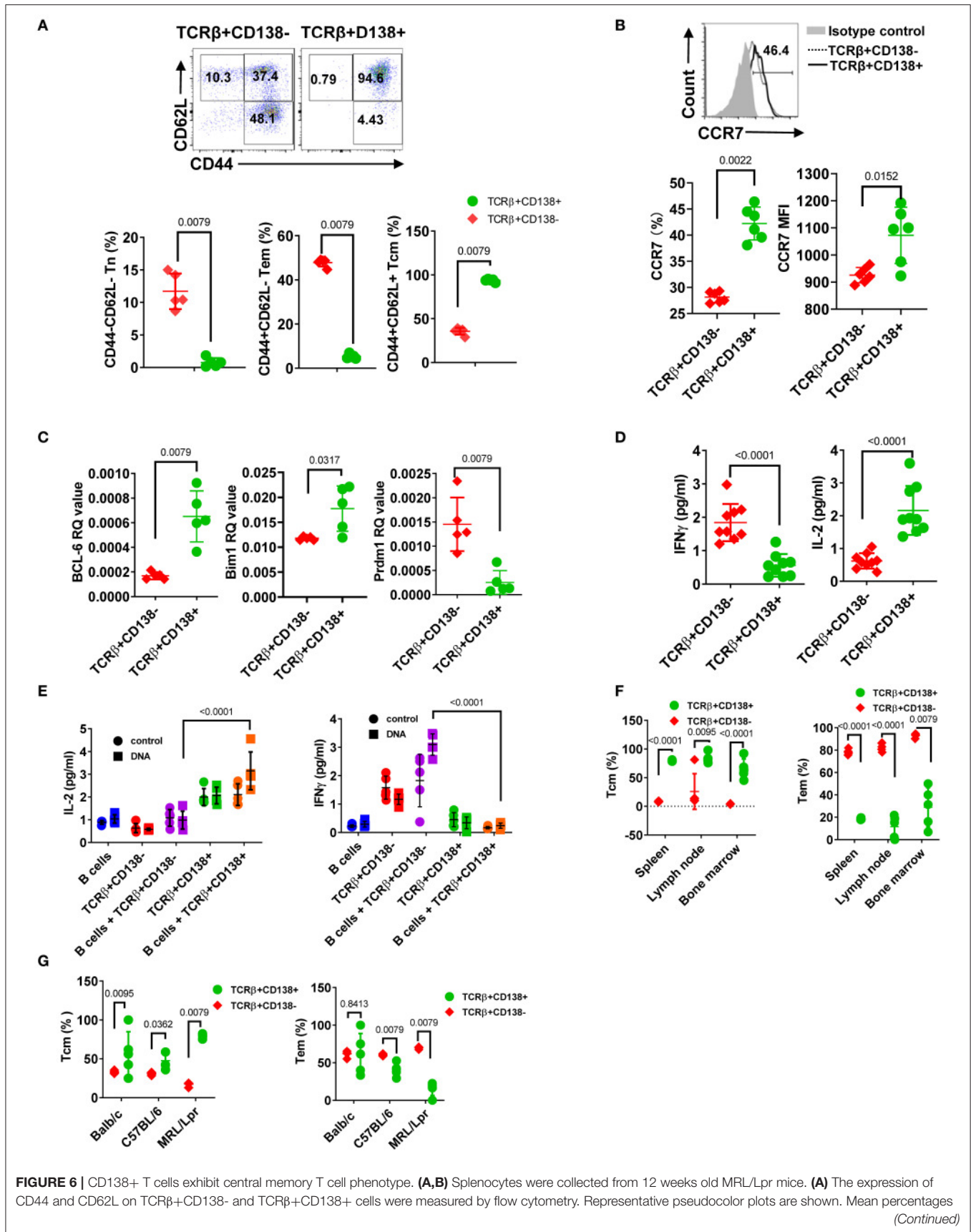


FIGURE 6 | CD138+ T cells exhibit central memory T cell phenotype. **(A,B)** Splenocytes were collected from 12 weeks old MRL/Lpr mice. **(A)** The expression of CD44 and CD62L on TCRβ+CD138- and TCRβ+CD138+ cells were measured by flow cytometry. Representative pseudocolor plots are shown. Mean percentages (Continued)

FIGURE 6 | \pm SD of five mice from two separate experiments are plotted. **(B)** Representative flow cytometry histogram of CCR7 expression on TCR β +CD138- and TCR β +CD138+ cells are shown. Mean \pm SD percentages and MFIs for CCR7 expression from six mice in two separate experiments are plotted. **(C)** Splenic TCR β +CD138+ and TCR β +CD138- cells were sorted from 12 weeks old MRL/Lpr mice and *BCL-6*, *Bim1*, and *Prdm1* mRNA were quantified by Q-PCR. Mean \pm SD values of five mice from two separate experiments are plotted. **(D)** Splenic TCR β +CD138+ and TCR β +CD138- cells were sorted from 12 weeks old MRL/Lpr mice and cultured with anti-CD3/CD28 antibodies overnight. Culture supernatant IFN γ and IL-2 levels were measured by ELISA. Mean \pm SD of nine mice from three independent experiments are plotted. **(E)** Splenic TCR β +CD138+, TCR β +CD138- cells were sorted from 12 weeks old MRL/Lpr mice and co-cultured with sorted B cells from the same mice in the presence of DNA for 3 days. Culture supernatant IFN γ and IL-2 levels were measured by ELISA. Mean \pm SD of five mice from two separate experiments are plotted. **(F)** Cells were collected from spleen, lymph nodes, and bone marrows of 12 weeks old MRL/Lpr mice. Frequencies of Tcm (CD44+CD62L+) and Tem (CD44+CD62L-) cells among TCR β +CD138- and TCR β +CD138+ cells were measured in flow cytometry. Mean percentages \pm SD of five mice from two independent experiments are plotted. **(G)** Splenocytes were collected from 12 weeks old Balb/c, C57BL/6, and MRL/Lpr mice. Frequencies of Tcm (CD44+CD62L+) and Tem (CD44+CD62L-) cells among TCR β +CD138- and TCR β +CD138+ cells were measured in flow cytometry. Mean percentages \pm SD of five mice from two independent experiments are plotted. Two tailed Mann-Whitney rank sum test was used to calculate statistical significance.

shown to be restricted to the gut epithelium, although they can expand to peripheral organs, such as lymph nodes and spleen when Fas ligand (*gld*) is ablated. Similar to C3H *gld* mice, a large population of CD138-expressing T cells accumulate in peripheral organs of Fas receptor mutant μ MT/Lpr, B6/Lpr (25) and MRL/Lpr mice (**Figure 1A**). As previously described, the majority of CD138+ T cells also express CD3 and B220 and are negative for CD4 and CD8 (dnT). Previous reports suggested that dnT cells derive from exhausted autoreactive CD8+ cells or continuously stimulated CD8+ cells (49, 50). Differing from these reports, our observations reveal that a substantial portion of the TCR β +CD138+ cells in MRL/Lpr mice are converted from CD4+ cells rather than CD8+ cells, as CD138+ cells emerged from *in vitro* cultured CD4+ cells, but not from CD8+ cells (**Figure 2D**). Interestingly, we found that the exposure of TCR β +CD138- cells to rapamycin severely reduced the increase in CD138 expression on these cells (**Supplemental Figure 2H**). Rapamycin was also reported to inhibit the generation of CD138-expressing plasma cells (33). These observations point to mTOR-mediated regulation of CD138 expression on lymphocytes. mTOR is also implicated in the pathogenesis of SLE, especially because systemic rapamycin administration alleviates lupus symptoms (51, 52). Also, inhibition of mTOR is shown to reduce the production of dnT cells and favors the expansion of therapeutic CD4+CD25+Foxp3+ Treg cells in lupus-prone mice and SLE patients (30–32). Further studies will be needed to test whether the beneficial effect of rapamycin in SLE is through its inhibition of CD138 expression on T cells.

Studies in normal mice or autoimmune-prone lpr mice have shown that dnT cells are able to dampen CD4+ and CD8+ T cell-mediated autoimmune responses both *in vitro* and *in vivo* (53, 54). Consistent with these studies, adoptively transferred TCR β +CD138+ T cells, of which the majority were CD4-CD8-cells, slowed down the disease progression in young MRL/Lpr mice when auto-antigens are not exposed. The TCR β +CD138+ cell-mediated suppression of disease progression is unlikely to be due to the apoptosis of host CD4+ and CD8+ T cells induced by the transferred cells as this mechanism requires functional Fas-FasL interaction (54). Suppression of dnT cell-mediated alloimmune responses are also attributed to elevated levels of perforin and granzyme B produced by these cells (55). This mechanism is also unlikely to be at play in the slowing down of disease progression by TCR β +CD138+ cells in young MRL/Lpr mice because we found comparable levels

of perforin and granzyme B production by TCR β +CD138+ and TCR β +CD138- cells (**Supplemental Figure 7**). Although the exact molecular mechanism and cascade of events need to be deciphered, we argue that the suboptimal proliferation capacity of TCR β +CD138+ cells as well as their diminished production of pathogenic cytokines, IL-17, TNF α , and IFN γ (56) may be responsible for the delay in disease progression afforded by these cells in young MRL/Lpr mice.

In active lupus patients or NZBxSWR mice, CD4+ as well as dnT cells augment the production of anti-dsDNA antibodies when they are co-cultured with oligoclonal autoreactive B cells (41, 57, 58). Consistent with these early observations, both TCR β +CD138+ and TCR β +CD138- cells enhanced plasma cell development and amplified autoreactive antibody production from MRL/Lpr mice B cells when auto-antigens were present. However, TCR β +CD138+ were more potent than TCR β +CD138- cells in activating autoreactive B cells both *in vitro* and *in vivo*. Interestingly, CD4-expressing TCR β +CD138+ cells were responsible for the activation of autoreactive B cells, despite comprising <20% percent of the total TCR β +CD138+ population. This rapid recall response upon antigen re-encounter is a typical characteristic of memory T cells (42, 47). Indeed, compared to TCR β +CD138- cells, over 90% of TCR β +CD138+ cells are CD44+CD62L+ Tcm cells. Although the other Tcm marker CCR7 is also higher on TCR β +CD138+ cells than on TCR β +CD138- cells, the frequency of CCR7- expressing TCR β +CD138+ cells is <50%. The discrepancy between the percentage of CD44+CD62L+ cells and CCR7+ cells may be due to possible loss of CCR7 expression on TCR β +CD138+ cells after repeated exposure to autoantigens, a phenomenon reported for CCR7+CD27+ memory T cells in lupus patients which lose CCR7 expression following repeated stimulation (13). Interestingly, clinical studies indicate that the expansion of dnT cells as well as the CD62L- CD197- effector-memory T cells and the CD62L+CD197+ central-memory cells are controlled by mTOR since rapamycin treatment decreased these populations in SLE patients (31, 32). The predominance of TCR β +CD138+ cells presenting with memory phenotype and the suppression of CD138-expression with rapamycin (**Supplemental Figure 2H**) in MRL/Lpr mice suggest that mTOR mediated expansion of CD138-expressing memory T cells may be associated with lupus pathogenesis in MRL/Lpr mice as well as in SLE patients. Of note, the Tcm characteristic of TCR β +CD138+ cells is independent of disease activity as TCR β +CD138+ cells from healthy MRL/Lpr

mice as well as different healthy mouse strains also exhibit similar phenotype. Thus, TCR β +CD138+ cells manifest Tcm phenotype, regardless of mouse strain and disease status.

Taken together, we have identified and characterized the phenotype of TCR β +CD138+ cells in MRL/Lpr mice and unveiled a pathogenic role for these cells in lupus disease. Both *in vivo* adoptive transfer experiments and *in vitro* co-culture experiments underpin the role of TCR β +CD138+ cells in activating autoreactive B cells when autoantigens are exposed. Although how CD138 expression renders TCR β + T cells more pathogenic is still enigmatic, the discovery of a novel Tcm subset with enhanced pathogenic properties may be useful in assessing and monitoring disease severity.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by US FDA, CBER Institutional Animal Care and Use Committee.

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AUTHOR CONTRIBUTIONS

LL and MA conceived the study and contributed to the manuscript writing. LL and KT did the laboratory work. LL, KT, and MA did the statistical analysis and contributed to the data analysis and interpretation. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2020.01569/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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