Soluble Syndecan I (CD138) Released by MRL/Lpr T Cells Enhances APRIL-Mediated Lupus B Cell Survival and Differentiation

LIU, LUNHUA and AKKOYUNLU, MUSTAFA

Laboratory of Bacterial Polysaccharides, Division of Bacterial Parasitic and Allergenic Products, CBER/FDA, Silver Spring, MD

Abstract

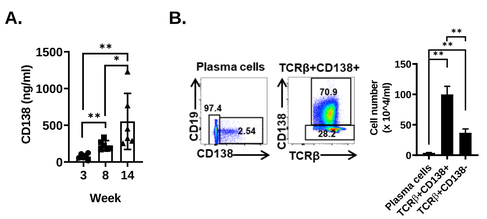
High level of soluble serum syndecan-1 (CD138), a heparan sulfate–bearing proteoglycan, in systemic lupus erythematosus (SLE) patients correlates with disease activity and lupus nephritis. Mechanisms responsible for the increase in serum CD138 and the immunopathological function of serum CD138 in lupus development remain poorly understood. In this study, recapitulating the findings in SLE patients, we found a sharp increase in serum CD138 levels parallel to the progression of disease in MRL/Lpr (lupus prone) mice. Although TCRβ+CD138+ T cells expand with age in MRL/Lpr mice, TCRβ+CD138- cells are the likely source of circulating CD138 as the transfer of TCRβ+CD138- cells, but not TCRβ+CD138+ cells, led to an increase in serum CD138 in the recipient mice. We found that CD138 expressed on lupus T cells was resistant to cleavage by MMP9 and collagenase, but it was very sensitive to trypsin. We also found high levels of trypsin production by TCRβ+CD138- cells. Suggesting a role for trypsin expressed by these cells in the elevated serum CD138 in lupus mice, trypsin produced by TCRβ+CD138- cells effectively cleaved CD138 from T cells. Interestingly, soluble CD138 was able to bind APRIL and enhance APRIL-mediated ERK activation and B cell differentiation. The ability of CD138 to potentiate APRIL-induced B cell differentiation was dependent on TACI expression, as the synergistic effect of APRIL and CD138 on plasma cell formation was strongly ablated on TACI deficient B cells. These findings reveal a role for soluble CD138 in B cell differentiation and autoreactive antibody production in MRL/Lpr mice. Understanding the mechanisms by which soluble CD138 is produced and how it functions may reveal novel druggable targets for lupus disease.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by hyperproduction of autoreactive antibodies that cause inflammation and multiple organ damage. This systemic pathological immune response, which involves both innate and adaptive immune system, is characterized by elevations of multiple cytokines in serum. Increased in serum levels of A Proliferation-Inducing Ligand (APRIL), B-cell-activating factor (BAFF), IFN-α, IFN-γ, IL-6, IL-12, IL-17 and TNF-α are found to be positively correlated with autoreactive antibody production, SLE Disease Activity Index (SLEDAI) scores, and organ involvement. In addition to the increase in inflammatory cytokines, SLE patients, but not rheumatoid arthritis patients, manifest with elevated serum levels of CD138 (syndecan-1). As a member of syndecan family of type I transmembrane proteoglycans, CD138 is composed of a core protein modified by heparan sulphate and chondroitin sulphate chains. Membrane bound CD138 has been shown to play an important role in wound healing, cell adhesion, and endocytosis. Like the other three members of syndecan family molecules, the intact ectodomain of CD138 is constitutively shed from cells and forms soluble CD138. soluble CD138 is also able to regulate a variety of molecule pathways that related to wound healing, cell proliferation and apoptosis. For example, increased soluble CD138 enhances the growth of myeloma tumors in vivo and promotes endothelial invasion and angiogenesis. Like APRIL and BAFF, high level of serum CD138 is also positively correlate with SLEDAI and anti-dsDNA antibody levels in lupus patient. Here, the origination of lupus serum CD138 and its immunopathological function were studied.

Results and Discussion

1. Activated TCRβ+CD138- cells release more soluble CD138 than TCRβ+CD138+ cells do



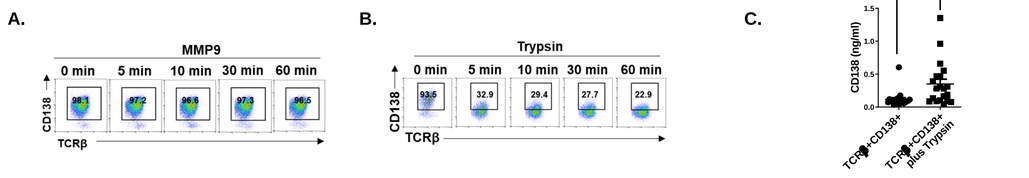
A, Serum CD138 levels in MRL/Lpr mice from different ages were measured by ELISA.

B, Blood were collected from 14 weeks old MRL/Lpr mice and plasma cells (CD19-CD138+), TCRβ+CD138+ cells, and TCRβ+CD138- cells were quantified in FACS.

C, Purified TCRβ+CD138- and TCRβ+CD138+ cells were activated and culture supernatant CD138 levels were measured by ELISA.

D, Purified TCRβ+CD138- or TCRβ+CD138+ cells were adoptively transferred into 8 weeks old MRL/Lpr mice and serum CD138 levels were determined 3 days later.

2. CD138 is cleaved from lupus T cells by trypsin

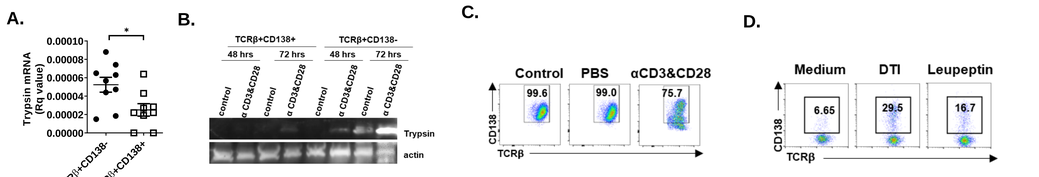


A, Purified TCRβ+CD138+ cells were treated with active MMP9 for indicated duration and CD138 levels on treated cells were measured by FACS.

B, Purified TCRβ+CD138+ cells were treated with active trypsin for indicated duration and CD138 levels on treated cells were measured by FACS.

C, Purified TCRβ+CD138+ cells were treated with trypsin for 5 minutes, and culture supernatant CD138 levels were measured by ELISA.

3. High intrinsic trypsin can constitutively shed CD138 from TCR+CD138- cells



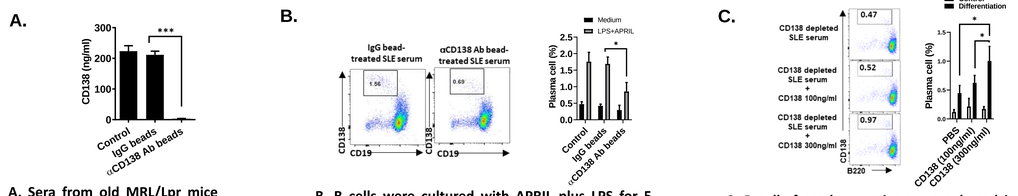
A, Trypsin mRNA levels were quantified in purified TCRβ+CD138+ and TCRβ+CD138- cells by Q-PCR.

B, Purified TCRβ+CD138+ and TCRβ+CD138- cells were treated with αCD3/CD28 Abs, after which trypsin expression was detected by Western blot assay.

C, Purified TCRβ+CD138+ cells were treated with αCD3/CD28 Abs for 48 hours and membrane CD138 expression was measured by FACS.

D, Purified TCRβ+CD138- cells were cultured in serum free medium and treated with trypsin inhibitors DTI and Leupeptin for 24 hours, after which percent of CD138 positive cells were measured by FACS.

4. CD138 in lupus mouse serum is responsible for B cell differentiation

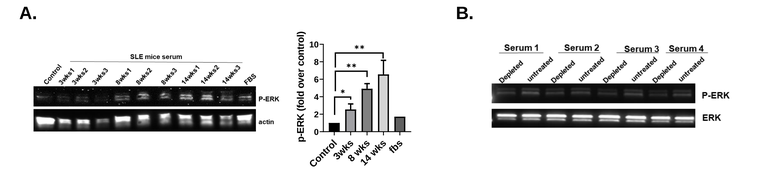


A, Sera from old MRL/Lpr mice were treated with IgG or CD138 Ab coupled Protein A/G beads. Remaining CD138 in the serum was quantified by ELISA.

B, B cells were cultured with APRIL plus LPS for 5 days in RPMI medium containing 10% lupus mouse serum (control) or lupus mouse serum treated with IgG beads or anti-CD138 Ab beads. After 5 days, plasma cells were quantified by FACS analysis.

C, B cells from lupus mice were cultured in CD138 depleted MRL/Lpr mice serum with or without exogenous CD138 and plasma cell development was assessed by FACS analysis after 5 days.

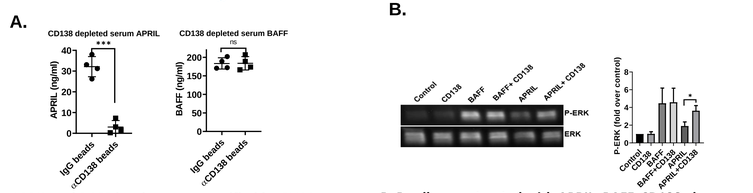
5. CD138 in lupus mouse serum is required for ERK activation in B cells



A, Lupus mouse B cells cultured in RPMI medium were left untreated (control), treated with 10% FBS or treated with 10% serum that was isolated from 3, 8 and 14 weeks old lupus mice (three separate mice for each age group) for 16 hours. ERK phosphorylation was assessed by Western blot analysis.

B, Lupus mouse B cells were treated with 10% untreated or CD138 depleted lupus mouse serum isolated from 14 weeks old mice for 16 hours. ERK phosphorylation was assessed by Western blot analysis.

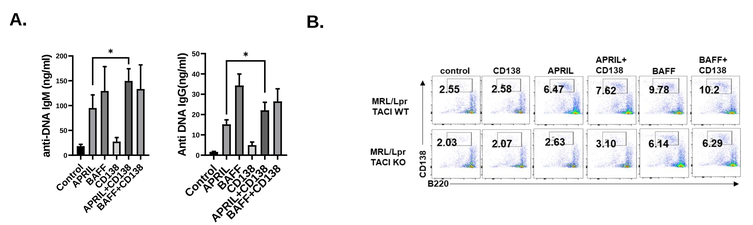
6. CD138 potentiates APRIL-induced ERK activation and B cell differentiation through TACI



A, APRIL and BAFF levels were quantified by ELISA after depletion of CD138 from sera of 14 weeks old lupus mice.

B, B cells were treated with APRIL, BAFF, CD138 alone or in combination. After 16 hrs, ERK phosphorylation was evaluated by Western blot analysis.

7. CD138 enhances APRIL-induced B cell differentiation and pathological antibody production through TACI



A. Lupus mouse B cells were treated with APRIL, BAFF, CD138 alone or in combination for 6 days, after which anti-DNA antibody levels in the culture supernatants were quantified by ELISA.

D. B cells isolated from wild-type or TACI-deficient MRL/Lpr mice were treated with CD138, APRIL, and BAFF alone or in combination for 6 days, after which plasma cells were quantified by FACS analysis.

Conclusion

Our findings indicate a regulatory role for serum CD138 in B cell differentiation and autoreactive antibody secretion in MRL/Lpr mice. CD138 expressed on lupus T cells is highly sensitive to trypsin cleavage. Increased trypsin expression by TCRβ+CD138- cells likely leads to cleavage of CD138 from cell membrane, which can contribute to the high level of soluble CD138 in lupus mice blood. Furthermore, soluble CD138 binds to APRIL and enhances APRIL-mediated ERK activation and B cell differentiation through TACI. Understanding the mechanisms of soluble CD138 production and function in MRL/Lpr mice can help improve the understanding of human lupus disease pathogenesis.