ADAM10-induced Cleavage of Semaphorin 4D from Eosnophils Contributes to Antibody Production in Bullous Pemphigoid

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INTRODUCTION

Lesional infiltration of eosinophils is a distinguishing histopathologic characteristic for bullous pemphigoid (BP). However, the actual contribution of eosinophils to the pathogenesis of BP is largely unknown. Semaphorin 4D (Sema4D) is constitutively expressed on granulocytes and plays important roles in B cell regulation, but its implication in BP remains obscure. Here, we described that eosinophils, marked as CD15⁺ granulocytes in patients with BP presented much lower expression of membrane-bound Sema4D (mSema4D) in both peripheral blood and blister fluid. Consistently, the levels of both lesional and circulating sSema4D were significantly elevated and well correlated with anti-BP180 antibody titer. Moreover, healthy-derived CD15⁺ granulocytes activated by PMA started spontaneous shedding of mSema4D and generating 120kDa-sized sSema4D into cell supernatants, which can be specifically blocked by ADAM10 inhibitor. Conformably, membrane-bound ADAM10 (mADAM10) was abundantly expressed on activated CD15⁺ granulocytes despite its absence before activation. Meanwhile, we found that soluble ADAM10 (sADAM10) treatment can directly cause mSema4D cleavage from CD15⁺ granulocytes. In line with *in vitro* findings, sADAM10 displayed a prominently high level in the blister fluid and mADMA10 was extensively expressed on CD15⁺ granulocytes from BP patients but nearly absent on those from healthy individuals. Furthermore, in patient-derived PBMCs, by promoting the differentiation of B cell into plasmablasts, sSema4D specifically boosted anti-BP180 antibody production in a time- and dose- dependent manners, which was attributed to CD72-mediated activation of Akt/NF- κ B p-65/Erk cascades in B cells. Our findings revealed a novel role of eosinophils in ADAM10-dependent Sema4D shedding that ultimately caused augmented pathogenic antibody and aggravated autoimmune responds, thus providing a new insight into the BP pathogenesis.

HYPOTHESIS

Sema4D participate in the BP development by modulating B cell activation and antibody production.

METHODS

Collection of peripheral blood, blister fluid, and skin tissue from patients with BP and normal control serum. Enzyme-linked immunosorbent assay, PBMC preparation and stimulation by sSema4D, Granulocyte preparation and B cell sorting, Isolation of blister infiltrated cells (BICs), Immunohistochemistry, Immunofluorescence and Western blotting, Intracellular phosphor-protein staining and Flow cytometry, Statistical analysis.

RESULTS

1. Sema4D levels are elevated in in serum, blister fluid, and lesions of patients with BP.

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Fig. 1. Levels of Sema4D were elevated in the serum, blister fluid, and lesions from patients with BP. Levels of Sema4D were elevated in the serum, blister fluid, and lesions from patients with BP. (a) Levels of sSema4D in serum from healthy individuals (n=11) and BP patients (n=32). (b) Levels of sSema4D in suction blister fluid from healthy individuals (n=5) and blister fluid from BP patients (n=13). Values in (a) and (b) presented as the mean $\pm$ SD. ND, not detectable. ***P < 0.001, vs. normal. (c) Immunohistochemistry staining of Sema4D in normal skin (NC) and BP lesions (BP). Bar, 100 μ m. (d) Immunofluorescence staining of Sema4D (green) in normal skin (NC) and BP lesions (BP). Nuclei were counterstained with DAPI (blue). Bar, 50 μ m. Images in (c) and (d) are representative of 5 normal skins and 5 BP lesions, respectively.

2. sSema4D levels in serum and blister fluid are closely related to disease severity and activity of patients with BP.

Fig. 2. sSema4D levels in serum and blister fluid are closely related to disease severity and activity of patients with BP. (a) Correlation of serum sSema4D levels with BPDAI. (b) Correlation of blister fluid sSema4D levels with BPDAI. (c) Correlation of serum sSema4D levels with the anti-BP180 antibody titer. (d) Correlation of blister fluid sSema4D levels with the anti-BP180 antibody titer. (e) Correlation of sSema4D level in serum and sSema4D level in blister fluid. (f) Expression of sSema4D in serum before and after treatment in patients with BP. ***P < 0.001. The adjusted R² and P value are plotted in each graph.

3. Sema4D promotes antibody production and B cell differentiation in PBMCs from BP patients.

Fig.3. Sema4D promotes antibody production and B cell differentiation in PBMCs from BP patients. (a) (b) Anti-BP180/230 antibody titers in supernatants of healthy-, BP180+ BP230- and BP180+ BP230+ patient-derived PBMCs unstimulated, stimulated with anti-CD3 antibody, anti-CD3 antibody plus Sema4D with or without adding anti-Sema4D antibody in day 3 for 7 days (n=5, 5, 4, respectively). (c) The ratio of CD19+CD138+ subset in CD19+ cells gated from unstimulated and stimulated patient-derived PBMCs and statistical analysis (n=8). Values are presented as the mean $\pm$ SD; **, CD3+Sema4D versus CD3; ##, CD3+Sema4D+Sema4D Ab versus CD3+Sema4D; *** P < 0.001, **or ## P < 0.01, # P < 0.05. NS, not significant.

4. Activation of NF- κ B/Akt/Erk cascades in B cells contribute to the Sema4D-induced anti-BP180 antibody production in patient-derived PBMCs.

Fig.4. Activation of NF- κ B/Akt/Erk cascades in B cells contribute to the Sema4D-induced anti-BP180 antibody production in patient-derived PBMCs.

(a) (b) p65, p-p65, Akt, p-Akt, Erk, and p-Erk expression detected by flow cytometry in CD19+ B cells gated from Sema4D-stimulated, unstimulated and isotype controls PBMCs from BP patients and their MFIs (n=5). (c) p65, p-p65, Akt, p-Akt, Erk, and p-Erk expression of sorted and gathered CD19+ B cells from 6 Sema4D-stimulated and unstimulated patient-derived PBMCs by Western blot. (d) Anti-BP180 antibody titers in supernatants of Sema4D-stimulated patient-derived PBMCs within B cells pre-treated with inhibitor of NF- κ B or Akt or Erk. Values in (a), (b) and (d) are presented as the mean $\pm$ SD ***P < 0.001, ** P < 0.01, *P < 0.05. NS, not significant.

5. ADAM10-induced CD15+ granulocytes shedding is responsible for the high levels of sSema4D in BP lesions and serum

Fig.5. ADAM10-induced CD15+ granulocyte shedding is responsible for the high levels of sSema4D in BP lesions and serum. (a)-(c) Histograms of membrane expression and MFIs of Sema4D on (a) CD3+ T cells and CD15+ granulocytes from BP patients and healthy controls (n=5). (b) CD15+ granulocytes before and after treatment of BP patients (n=5). (c) CD15+ granulocytes from BP blood, BP blister fluid, and healthy blood (n=5). (d) sSema4D level in supernatants of granulocytes treated with indicated enzymes, histograms and MFI of membrane expression of Sema4D on ADAM10-treated CD15+ granulocytes (n=5).

Fig.6. ADAM10-induced CD15+ granulocyte shedding is responsible for the high levels of sSema4D in BP lesions and serum. (a) sSema4D level in supernatants of PMA-activated CD15+ granulocytes followed with or without ADAM10/ADAM17/ MT-MMP1 inhibitor (n=5). (b) Histograms and MFIs of membrane expression of Sema4D on PMA-activated CD15+ granulocytes followed with or without ADAM10/ADAM17/ MT-MMP1 inhibitor (n=5). (c) The ratio of ADAM10+ CD15+ subset in CD15+ granulocytes activated by PMA at indicated time points and statistical analysis(n=5). (d) ADAM10 levels in suction blister fluid and blister fluid (n=5). (e) The ratio of ADAM10+ CD15+ subset in CD15+ granulocytes of healthy individuals and BP patients and statistical analysis (n=5). Values are presented as the mean $\pm$ SD ***P < 0.001, ** P < 0.01. NS, not significant.

CONCLUSION

Membrane-bound ADAM10 expressed on activated CD15⁺ granulocytes in circulation, along with soluble ADAM10 in local lesions, cause Sema4D cleavage from the membrane of CD15⁺ granulocytes and result in high level of circulating sSema4D. Elevated sSema4D subsequently triggers the activation of NF- κ B/Akt/Erk signaling in B cells and promote B cell differentiated into plasmablasts, which enhances the antibody production and amplify autoimmune responses.

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