主論文の要旨

Pristane-induced Granulocyte Recruitment Promotes Phenotypic Conversion of Macrophages and Protects Against Diffuse Pulmonary Hemorrhage in Mac-1 Deficiency

（プリスタンにより誘導される顆粒球導入は、
Mac-1 欠損下においてマクロファージの形質転換を誘導し、
び慢性肺胞出血に対して保護的役割を担う）

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Introduction

Diffuse pulmonary hemorrhage (DPH) is an uncommon but critical complication of systemic lupus erythematosus (SLE). Its prevalence ranges between <2% and 5.4% and the mortality rate ranges from 23% to 90%. Spontaneous developments can be rapidly unfavorable, with death occurring in the 48 h following the onset of the first symptoms. Peritoneal administration of 2,6,10,14-tetramethylpentadecane (pristane, TMPD) can recapitulate a lupus-like syndrome in mice, which can develop into DPH within a few weeks, especially in C57BL/6 mice. Mac-1 (CD11b/CD18), a leukocyte adhesion molecule, is known to play a role in inflammation by regulating migration of leukocytes into injured tissue. In this study, we focused on innate immunity in pristane-induced SLE and explored the critical role of granulocytes for phenotypic conversion of macrophages to immunoregulatory cell types, which is tightly connected to amelioration of pristane-induced DPH.

Methods

Animal model: C57BL/6 WT and Mac-$1^{-/-}$ mice received a single 0.5mL i.p. injection of pristane.

Evaluation of DPH severity: The percentage of lung with hemorrhage was estimated and assigned one of the following scores in the left lobe: 0, no hemorrhage; 1, 0%–25%; 3, 25%–50%; 3, 50%–75%; and 4, 75%–100%. Each lobe was randomly cut into 5 sections. The scores were evaluated independently by two observers in a blinded manner and the average scores for each animal were used for analysis.

Flow cytometry analysis: Single-cell suspensions from lungs and peritoneal cavities were washed in MACS buffer. After blockade of Fc receptors with rat anti-mouse CD16/CD32 mAb, cells were stained with the corresponding antibody mixtures. The following mAb were used: APC-labeled anti-F4/80, Alexa Fluor 488-labeled anti-Mannose receptor, APC-labeled anti-Ly6G, and PE-labeled anti-siglec-F. Cells were analyzed using a FACSCanto flow cytometry system. All the data were analyzed using the FlowJo software.

Cytokine analysis: The concentrations of TNFα, IL-4, IL-6, and IL-13 in peritoneal lavage fluids were measured using ELISA kits purchased from R&D Systems.

Selective depletion of granulocyte subsets: For neutrophil depletion, mice were treated by i.v. injection with 50 μg anti-Ly6G or its isotype control IgG2a on alternating days following pristane challenge. For eosinophil depletion, mice were administered with 20 μg anti-IL5 or its isotype control IgG1 i.p. injection one day before pristane treatment. Efficacies of neutrophil or eosinophil depletion were confirmed by flow cytometry in all experimental animals.

Preparation of adoptive cell transfer of M1 and M2 macrophages: For M1 polarization, BMDMs (Bone marrow derived macrophages 5×10^5/mL) were cultured with MCM (RPMI 1640 medium-FBS containing 20% v/v L929 cell-conditioned medium) for 7 days, and then stimulated by LPS (4 μg/mL) and IFN-γ (20 ng/mL) in RPMI-10%FBS for 2 days. For M2 polarization, BMDMs (5×10^5/mL) were cultured with MCM for 3 days, and subsequently incubated with IL-4 and IL-13 (20 ng/mL) for 4 days.

Statistical analysis: Data are expressed as the mean ± SEM unless otherwise indicated. Statistical significance was tested with an unpaired Student's t-test using Prism 5. For multiple comparisons, one-way ANOVA with Bonferroni correction was used. A *P*-value of <0.05 was considered to be statistically significant.

Results

Refer to the next pages.

Discussion

DPH is a rare, life-threatening complication in patients with SLE. In the current study, we clearly demonstrated the role of the leukocyte integrin Mac-1 in promoting pristane-induced DPH, as Mac-$1^{-/-}$ mice showed a significantly reduced prevalence of DPH than WT animals, as evidenced by decreased mortality and mild pathology. Analysis of the peritoneal lavage on day 5 and day 10 after pristane treatment revealed increased numbers of eosinophils and alternatively activated macrophages but decreased numbers of neutrophils and classically activated macrophages in Mac-$1^{-/-}$ mice compared to WT. Enhanced production of IL-4 and IL-13, both key mediators of macrophage polarization toward the MMR^+ phenotype, was observed in the peritoneal cavity of Mac-$1^{-/-}$ mice. Depletion of neutrophils and eosinophils or adoptive transfer of M1 macrophages resulted in the exacerbation of pristane-mediated DPH in both WT and Mac-$1^{-/-}$ mice. These results indicated that protective effects of neutrophils were critical during the early phase of pristane exposure. Eosinophils were critical for IL-4 secretion and subsequently modulated M2 macrophage polarization. Moreover, peritoneal transfer of $F4/80^{high}MMR^+$ M2 macrophages successfully reduced the prevalence of DPH in WT mice.

Conclusion

In conclusion, abundant recruitment of granulocytes in the Mac-1 deficient peritoneal cavity resulted in impaired inflammatory M1 macrophage and enhanced M2 macrophage accumulation. This M2-dominant macrophage polarization in the peritoneal cavity was closely associated with impaired pro-inflammatory cytokines in Mac-1 deficiency, but neutrophils and eosinophils played different roles in the phenotypic conversion of macrophages into M2 cells.