学位論文 (要約)

T-cell dependent reactive granulopoiesis is associated with neutropenia-induced alteration of gut microbiota

(好中球減少時の T 細胞依存性反応性顆粒球造血は, 好中球減少によって変化した腸内細菌叢によって促進される)

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【Introduction】

Granulopoiesis of the bone marrow is regulated in response to the demand for neutrophils. Severe bacterial infections, for example, induce "emergency granulopoiesis" in which granulocytes are increased several-fold above steady state to eliminate pathogens. Reactive granulopoiesis, on the other hand, refers to increased neutrophils in the absence of microbial infection, induced by an increase in prolonged inflammatory stimuli or by neutropenia induced by hematopoietic stem cell transplantation (SCT) or chemotherapy. Emergency granulopoiesis has been reported to be dependent on granulocyte colony-stimulating factor (G-CSF) production, which is facilitated by pathogen-associated molecular patterns, but the mechanism by which neutropenia induces reactive hematopoiesis remains unclear. Since reactive granulopoiesis contributes significantly to the safety of hematopoietic stem cell transplantation and cancer chemotherapy in clinical practice, elucidating the mechanism of reactive granulopoiesis is important for further refining these therapies. Recent studies have demonstrated that the gut microbiota plays an important role in the development of granulocyte formation in infants after birth. In this study, we investigated the mechanism by which neutropenia after SCT or chemotherapy induces reactive granulopoiesis using mouse models. The results of this study revealed that neutropenia enhances IL-17A production by T cells in a gut microbiota-dependent manner and promotes granulopoiesis. Furthermore, we found that prolonged neutropenia changes the composition of the intestinal microflora and further promotes IL-17A production and granulopoiesis.

【Materials&Methods】

In the SCT model, recipient B6 mice were lethally irradiated and infused with 7,500 lineage$^-$ sca-1$^+$ c$^-$ kit$^+$ cells (LSK) and 20,000 granulocyte-macrophage progenitors (GMP) isolated from B6-CD45.1 mice. In the chemotherapy model, B6 mice were injected with 200 mg/kg 5-fluorouracil (5-FU) on day 0. For gut decontamination, recipients were administered in drinking water with ampicillin, streptomycin, and vancomycin for 7 days prior to SCT, and neutrophils (CD11b$^+$ Ly6G$^+$) in peripheral blood were evaluated for up to 4 weeks following transplant. In addition, hematopoietic progenitor cells such as LSKs, common myeloid progenitor cells (CMPs) and granulocyte-monocyte progenitors (GMPs) were quantified in the bone marrow after SCT using flow cytometry.

【Results】

First, we found neutropenia persisted for more than 10 days after SCT or 5-FU injection, and plasma levels of IL-17A and G-CSF significantly increased on day+18 after SCT or day+14 after chemotherapy. Therefore, we investigated the role of IL-17A in neutrophil recovery after SCT using B6-IL-17A-deficient (*IL17A*$^{-/-}$) mice as recipients. We found that plasma levels of IL-17A and G-CSF, and bone marrow GMP

counts after SCT were significantly lower in *IL17A*$^{-/-}$ recipients compared to *WT* recipients on day+18, indicating that IL-17A plays a critical role in granulopoiesis after SCT. When B6-RAG1-deficient (*RAG1*$^{-/-}$) mice were used as recipients, plasma levels of IL-17A were significantly decreased, and neutrophil recovery was significantly delayed compared to *WT* recipients, suggesting that T cells or B cells play an important role in IL-17A elevation and reactive granulopoiesis after SCT. Injection of 6×10^6 T cells purified from naive *WT* B6 mice into *RAG1*$^{-/-}$ or *IL17A*$^{-/-}$ recipients on day 0 of SCT significantly accelerated neutrophil recovery, confirming the critical role of T-cell production of IL-17A in reactive granulopoiesis following SCT. Next, we explored the role of intestinal microbiota in reactive granulopoiesis after SCT. Gut decontamination prevented the increase in plasma levels of IL-17A and G-CSF after SCT in *WT* recipients and significantly delayed neutrophil recovery. 16S-rRNA sequencing of fecal samples showed significant changes in the intestinal microbiota on day 10 after SCT compared to naive mice, with significant increases in bacteria of the *Ruminococcaceae* family and the genus *Ruminococcaceae UCG-014*. These changes in the flora were also seen after 5-FU administration, but not after bone marrow transplantation, where neutrophil engraftment is seen rapidly within a week, suggesting that they are caused by prolonged neutropenia. Finally, gut-decontaminated recipients underwent fecal microbiota transplantation (FMT) on day+14 after SCT, using fecal suspensions collected from SCT recipients on day +10 or those collected from naïve mice. Plasma levels of IL-17A, absolute numbers of GMPs in the bone marrow, and neutrophil numbers in the peripheral blood were significantly increased in the recipients underwent FMT from SCT recipients compared to those received FMT from naive mice. These results indicate that prolonged neutropenia induced the gut microbiota which can efficiently promote reactive granulopoiesis after SCT.

【Discussion】

The intestinal microbiota was altered by prolonged neutropenia but not after short-term neutropenia. This study demonstrates that neutrophil depletion induces changes in the gut microbiota, that stimulates T-cell production of IL-17A and promotes reactive granulopoiesis. Gut microbiota of the *Ruminococcaceae* family have been shown to be associated with neutrophil engraftment after clinical SCT, and results from the current study revealed novel crosstalk between bone marrow granulopoiesis and gut microbiota (Schluter et al., Nature, 2020). The development of novel methods of transplantation and chemotherapy that can retain granulopoiesis-stimulating bacteria may lead to the development of safer SCT and chemotherapy expecting early neutrophil recovery.

【Conclusion】

In this study, we demonstrated that prolonged neutropenia leads to alteration of gut microbiota and stimulates reactive granulopoiesis by promoting IL-17A production from T cells.