学位論文内容の要旨
（Summary of dissertation）

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学 位 論 文 題 名（Title of dissertation）

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)

Background and Purpose:

Granulopoiesis in the bone marrow adjusts to the cellular output demand. Severe bacterial infection induces “emergency granulopoiesis” that can increase granulocytes several-fold above the steady-state level to eliminate infectious agents. The term “reactive granulopoiesis” refers to enhanced granulopoiesis in the absence of active microbial infection that could be induced by neutropenia after hematopoietic stem cell transplantation (SCT) or chemotherapy for cancer. While it was reported that emergency granulopoiesis is dependent on granulocyte colony-stimulating factor (G-CSF) production promoted by pathogen-associated molecular patterns, the mechanism by which neutropenia induces reactive granulopoiesis remains to be clarified. Since reactive granulopoiesis greatly contributes to the safety of clinical hematopoietic SCT and chemotherapy for cancer, it is important to elucidate the precise mechanism underlying reactive granulopoiesis to further refine these procedures. Recent studies demonstrated that the intestinal microbiota plays a critical role in the development of granulopoiesis in infants after birth. In the current study, we studied the mechanism by which neutropenia induces granulopoiesis after SCT or chemotherapy depending on the microbiota-dependent T cell production of IL-17A.

Materials and Methods:

In models of SCT, recipient B6 mice were lethally irradiated and injected with 7,500 lineages of negative lineage$^-$sca-1$^+$c-kit$^+$ cells (LSKs) plus 20,000 granulocyte-macrophage progenitors (GMPs) purified from congenic B6-CD45.1 mice on day 0. In chemotherapy models, recipient B6 mice were injected with 200 mg/kg 5-fluorouracil (5-FU) on day 0. For gut decontamination, recipients were administered a combination of ampicillin, streptomycin, and vancomycin in drinking water daily from day -7 of SCT, and the number of neutrophils identified as CD11b$^+$ Ly6G$^+$ cells in the peripheral blood was evaluated for up to 4 weeks following SCT. Furthermore, flowcytometric analyses of hematopoietic progenitor cells, such as LSK, GMP, CMP, and MEP, in the bone marrow were performed after SCT.

Results:

First, we observed that plasma levels of IL-17A and G-CSF were significantly elevated on day +18 after SCT. Thus, we studied the role of IL-17A in neutrophil recovery after SCT using B6-IL-17A-deficient (*IL17A*$^{-/-}$) mice as recipients. We observed that the elevation of the plasma levels of IL-17A and G-CSF were abrogated and neutrophil recovery was significantly delayed in *IL17A*$^{-/-}$ recipients compared with wild type (*WT*) recipients. The numbers of bone marrow granulocyte-macrophage progenitors (GMPs) after SCT were also significantly lower in *IL17A*$^{-/-}$ recipients compared than in *WT* recipients, suggesting that IL-17A plays a critical role in granulopoiesis after

SCT. When B6-RAG1-deficient (*RAG1*$^{-/-}$) mice were used as the recipients, the elevation in the plasma IL-17A levels was significantly decreased and neutrophil recovery was significantly delayed compared with those in *WT* recipients, indicating a critical role of host T cells in IL-17A production and granulopoiesis after SCT. When *RAG1*$^{-/-}$ or *IL17A*$^{-/-}$ recipients were injected with 6×10^6 T cells purified from naïve *WT* B6 mice on day 0 of SCT, neutrophil recovery was significantly enhanced, further confirming the critical role of T cells and IL-17A in granulopoiesis following SCT. Furthermore, we explored the role of gut microbiota in granulopoiesis following SCT. Gut decontamination abrogated the elevation of the plasma levels of IL-17A and G-CSF and significantly delayed neutrophil recovery in *WT* recipients following SCT. 16S-rRNA sequencing of fecal samples demonstrated that a significant alteration in the gut microbiota following SCT and fecal microbiota transplantation 14 days following SCT significantly promoted neutrophil recovery in decontaminated SCT recipients.Then, we demonstrated that neutrophil recovery following chemotherapy (5-FU) was delayed in gut decontaminated mice or *IL17A*$^{-/-}$ mice, suggesting that neutrophil recovery following chemotherapy was also dependent on IL-17A secretion in response to microbiota. Next, we demonstrated that *Ruminococcaceae* (*Ruminococcaceae UCG-014*), an uncultured genus-level group, significantly increased after prolonged neutropenia but abrogated after unmanipulated BMT, suggesting that prolonged neutropenia increased the numbers of this bacterial genus. Finally, we performed a FMT model to gut-decontaminated SCT recipients underwent fecal microbiota transplant (FMT) on day +14 following SCT using fecal suspension harvested from SCT recipients who were not administered antibiotics on day +10. The plasma levels of IL-17A, level of neutrophil recovery in the peripheral blood, and number of GMPs in the bone marrow were significantly increased in these recipients, which underwent FMT from SCT recipients, compared with the FMT recipients from naïve control mice. These results indicate that intestinal microbiota promoted reactive granulopoiesis following SCT.

Discussion:

Prolonged neutropenia altered the gut microbiota, which did not alter following a short period of neutropenia. This study demonstrated that the depletion of neutrophils stimulated T-cell production of IL-17A in a microbiota-dependent manner, which promoted reactive granulopoiesis. Following SCT, the T-cell production of IL-17A was upregulated. Neutrophil recovery was significantly delayed in IL-17A-deficient and T cell-deficient mice, but the adaptive transfer of wild-type T cells facilitated neutrophil engraftment. Gut decontamination with oral antibiotics suppressed IL-17A production, leading to delayed neutrophil recovery following SCT and 5-FU injection. In both models, *Ruminococcaceae UCG-014* was significantly increased in the gut microbiota. The transplantation of fecal microbiota collected from SCT recipients, but not that from naïve mice, promoted neutrophil recovery in the antibiotic-treated SCT recipients, suggesting that neutropenia-induced microbiota has a potential to stimulate reactive granulopoiesis.

Conclusion:

Our study reveals a novel role of gut microbiota in reactive granulopoiesis during prolonged neutropenia, and it demonstrates that the gut microbiota enhances granulopoiesis by stimulating IL-17A production by T cells.