

## 学位論文内容の要旨

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### 学位論文題名

Studies on inhibitory interaction between graft-derived and reconstituted T cells involves murine chronic graft-versus-host disease  
(慢性移植片対宿主病における移植片由来および再構築由来 T 細胞の相互作用に関する研究)

#### 【Background and Objectives】

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative therapy for a variety of hematopoietic disorders. However, allogeneic T cell responses in which donor T cells recognize the host tissues as non-self and attack them result in graft-versus-host disease (GVHD). GVHD can be divided into two distinct syndromes, acute and chronic GVHD. The pathogenesis of chronic graft-versus-host disease (cGVHD) remains elusive. Donor T cells in the cGVHD recipients are comprised of two distinct T cell populations; graft-derived T cells ( $T_G$ ) and hematopoietic stem cell-derived reconstituted T cells ( $T_{HSC}$ ). Because cGVHD develops following the reconstitution of  $T_{HSC}$ , which underwent aberrant thymic negative selection that occurred during acute GVHD,  $T_{HSC}$  have been considered to be pathogenic. However, there is clinical evidence that older patients with poor thymopoiesis are at increased risk of cGVHD, which suggests that  $T_{HSC}$  is not prerequisite for cGVHD but  $T_G$  also contribute to cGVHD. Consistent with this theory, previous reports have shown that  $T_G$  persist throughout the chronic phase in cGVHD mice with poor  $T_{HSC}$  reconstitution. However, it remains unclear whether these persistent  $T_G$  sustain effector function and contribute to cGVHD. Host-type histocompatibility antigens persist for a long time following allo-HSCT, which may deprive allo-reactive persistent  $T_G$  of effector function by mechanisms of exhaustion or replicative senescence, similar to the process that occurs in the chronic viral infection or cancer. Thus, with the contributions of  $T_G$  and  $T_{HSC}$  being limited in supply or by a loss of function, respectively, the extent to which each of these T cell populations is responsible for the pathogenesis of cGVHD has remained unclear. One of the major hurdles to understanding the contribution of  $T_G$  and  $T_{HSC}$  is a lack of murine models that replicate the course of human cGVHD, specifically, models with autoimmune-like pathological features that meets clinical diagnostic criteria and which develops as  $T_{HSC}$  numbers increase. Here, we established a clinically relevant murine model of cGVHD in order to characterize the reciprocal regulation between  $T_G$  and  $T_{HSC}$  in cGVHD by selective T cell depletion.

#### 【Material and Methods】

C3H.Sw (H2b) recipients received 9Gy total body irradiation (TBI) before transfer of T cell depleted BM (TCD BM) with (“cGVHD group”) or without (“BMT group”) spleen  $CD4^+$  and  $CD8^+$  T cells from MHC minor-mismatched B6 (H2b) donors. After 9 weeks, histological analysis was performed on cGVHD-affected organs (lung, liver, skin and salivary gland) according to NIH criteria. The kinetics and function of  $T_G$  and  $T_{HSC}$  in affected-organs and secondary lymphoid organs (SLOs) were examined using congenic systems. An anti-Thy1.2 monoclonal antibody (mAb) was used to selectively deplete  $T_G$  or  $T_{HSC}$  during the chronic phase of disease.

### **【Results】**

In the [B6 → C3H.SW] cGVHD model, cGVHD mice developed pathology that recapitulates human cGVHD in the liver, lung, skin and salivary glands. To determine the extent of T<sub>HSC</sub> reconstitution, we examined the reconstitution of T<sub>HSC</sub> by using CD45.1 / CD45.2 congenic system. The number of CD4<sup>+</sup> CD8<sup>+</sup> double positive (DP) thymocytes of T<sub>HSC</sub> in cGVHD group was significantly lower than in the BMT group from day 35 to day 63. Delay and impairment of T<sub>HSC</sub> reconstitution was observed. Total T cell number in the liver and lung was higher in cGVHD group than in BMT group. Time course analysis revealed that detectable numbers of T<sub>HSC</sub> appeared in the liver, lung and spleen from day 21 after HSCT, T<sub>G</sub> outnumbered T<sub>HSC</sub> for duration of our experiment. We next examined whether the persisted T<sub>G</sub> in the affected organs at day 63 are exhausted or still functional. T<sub>G</sub> included a large proportion of PD-1<sup>+</sup> exhausted or KLRG-1<sup>+</sup> replicative senescent T cells, but also included a functional population with the potential to proliferate and produce inflammatory cytokines such as IFN $\gamma$  and TNF $\alpha$ . To determine the contribution of persistent T<sub>G</sub> and T<sub>HSC</sub> to cGVHD pathogenesis, we performed selective depletion of these cells in the chronic phase. Selective T<sub>G</sub> depletion failed to block cGVHD development because of compensatory proliferation and activation of T<sub>HSC</sub> in affected organs. On the other hand, selective T<sub>HSC</sub> depletion resulted in activation of T<sub>G</sub> without increase leading to lethal exacerbation of cGVHD, indicating that even a small number of T<sub>HSC</sub> play a critical role in inhibiting T<sub>G</sub> activation in the acute-to-chronic transition phase.

### **【Discussion】**

Our cGVHD murine model recapitulates the important feature of human cGVHD such as salivary gland and lung damage with concomitant immunodeficiency. In addition, the biphasic development of GVHD symptoms recapitulated the acute-to-chronic transition that occurs in the course of human cGVHD course.

In our cGVHD model, a large number of T<sub>G</sub> persisted in cGVHD-affected organs up to day 63 after allo-HSCT with an unexpected predominance of T<sub>G</sub>. The majority of T<sub>G</sub> persisting in the affected organs had an effector phenotype, which is distinct from the memory stem cell population. PD-1<sup>+</sup> KLRG-1<sup>+</sup> replicative senescent T<sub>G</sub> may be involved in the cellular immunopathogenesis of cGVHD. Unlike the basal maintenance of memory T cells, a significant proportion of T<sub>G</sub> were actively proliferating in the liver and lung even at day 63. These results suggest that the number of T<sub>G</sub> is controlled by active proliferation and cell death as well as by homeostatic cytokines. The extent of the involvement of microenvironment-derived allo-antigens and cytokines remains to be elucidated.

Reconstitution of T<sub>HSC</sub> is markedly delayed and suppressed in the presence of GVHD. T<sub>G</sub> may suppress T<sub>HSC</sub> reconstitution by impairing primary lymphoid tissues. In this study, depletion of T<sub>G</sub> resulted in a rapid increase of T<sub>HSC</sub> in the liver and lung. This finding points to the possible existence of a niche that antigenic signals and survival factors to pathogenic T cells of T<sub>G</sub> or T<sub>HSC</sub> origin. Such a “pathogenic T cell niche” might have a fixed pool capacity, meaning that T<sub>G</sub> and T<sub>HSC</sub> compete with each other for space in the niche during cGVHD. Our observation that the depletion of a small number of T<sub>HSC</sub> did not influence the number of T<sub>G</sub> in cGVHD-affected organs is consistent with the replicative senescent phenotype of T<sub>G</sub> and the hypothesis that the size of the pathogenic T cell niche is limited.

### **【Conclusion】**

We have characterized the cellular mechanisms underlying the maintenance of pathogenic T cells in a clinically relevant cGVHD model. Both T<sub>G</sub> and T<sub>HSC</sub> with the potential to proliferate and produce inflammatory cytokines infiltrated cGVHD-affected organs. However, a reciprocal regulatory interaction determined the balance between the number and activity of T<sub>G</sub> and T<sub>HSC</sub>, and thus maintains the pathogenic T cell pool.