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博士の専攻分野の名称 博士（医 学） 氏 名 小杉 瑞葉

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_{HSC} reconstitution, we examined the reconstitution of T_{HSC} by using CD45.1 / CD45.2 congenic system. The number of CD4⁺ CD8⁺ double positive (DP) thymocytes of T_{HSC} in cGVHD group was significantly lower than in the BMT group from day 35 to day 63. Delay and impairment of T_{HSC} 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_{HSC} appeared in the liver, lung and spleen from day 21 after HSCT, T_G outnumbered T_{HSC} for duration of our experiment. We next examined whether the persisted T_G in the affected organs at day 63 are exhausted or still functional. T_G included a large proportion of PD-1⁺ exhausted or KLRG-1⁺ replicative senescent T cells, but also included a functional population with the potential to proliferate and produce inflammatory cytokines such as IFN γ and TNF α . To determine the contribution of persistent T_G and T_{HSC} to cGVHD pathogenesis, we performed selective depletion of these cells in the chronic phase. Selective T_G depletion failed to block cGVHD development because of compensatory proliferation and activation of T_{HSC} in affected organs. On the other hand, selective T_{HSC} depletion resulted in activation of T_G without increase leading to lethal exacerbation of cGVHD, indicating that even a small number of T_{HSC} play a critical role in inhibiting T_G 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_G persisted in cGVHD-affected organs up to day 63 after allo-HSCT with an unexpected predominance of T_G . The majority of T_G persisting in the affected organs had an effector phenotype, which is distinct from the memory stem cell population. PD-1⁺ KLRG-1⁺ replicative senescent T_G may be involved in the cellular immunopathogenesis of cGVHD. Unlike the basal maintenance of memory T cells, a significant proportion of T_G were actively proliferating in the liver and lung even at day 63. These results suggest that the number of T_G 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_{HSC} is markedly delayed and suppressed in the presence of GVHD. T_G may suppress T_{HSC} reconstitution by impairing primary lymphoid tissues. In this study, depletion of T_G resulted in a rapid increase of T_{HSC} 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_G or T_{HSC} origin. Such a “pathogenic T cell niche” might have a fixed pool capacity, meaning that T_G and T_{HSC} compete with each other for space in the niche during cGVHD. Our observation that the depletion of a small number of T_{HSC} did not influence the number of T_G in cGVHD-affected organs is consistent with the replicative senescent phenotype of T_G 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_G and T_{HSC} 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_G and T_{HSC} , and thus maintains the pathogenic T cell pool.