論 文 の 内 容 の 要 旨

論文題目

Quantitative analysis of homeostatic T cell development in the thymus

（胸腺におけるT細胞生成プロセスの恒常性に関する定量生物学的研究））

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T cells play vital roles in the immune system. A diverse T cell receptor (TCR) repertoire is essential to defend the body against numerous kinds of pathogens, and a variety of T cell phenotypes shape sophisticated immune reactions to eliminate and remember pathogens while keeping cells of the body undamaged. The diverse TCR repertoire and the functional phenotype composition, along with a large number of cells, owe their origin to the thymus. T cell development in the thymus is thus essential for the immune system. Although we have accumulating evidence that T cell development is a homeostatic process in various aspects, it remains elusive how the homeostatic T cell development is maintained. In this thesis, we quantitatively studied homeostasis of population size, TCR repertoire diversity, and intracellular signaling network in thymic T cell development by utilizing mathematical modeling and high throughput sequence analysis.

Thymocytes, immature T cells in the thymus, differentiate in a stepwise manner. The differentiation process of a significant fraction of T cells is divided into three steps; CD4-CD8- double negative (DN), CD4+CD8+ double positive (DP), and CD4+CD8- single positive (SP4) stages. This differentiation process is regulated by mutual interactions with two types of thymic epithelial cells (TECs), cortical and medullary TECs (cTECs and mTECs). It remains unclear

how the mutual interactions contribute to the stable population size of thymocytes and TECs. To address this problem, we construct a mathematical model for population dynamics of thymocytes and TECs during the recovery from irradiation to investigate the relationship between cell-to-cell interactions and their population sizes.

The mathematical model can reproduce experimentally observed time courses of cell population size. We can also infer a regulatory network of thymocytes and TECs from the model, and reveal its role for the homeostasis of thymic cell populations. We find that DN thymocytes and cTECs form a negative feedback loop, which results in the overshooting dynamics of population sizes thereof. We further analyze the detail differentiation process of DN thymocytes because the DN stage is subdivided into DN1, DN2, DN3, and DN4 stages in the order of differentiation, and they interact with cTECs in different ways. We modify the model to include more detailed differentiation stages from DN1 to DN4. The detailed model reproduces the dynamics of subpopulations of DN1 to DN4. We also obtain the DN population dynamics almost the same as the proposed model by summing up DN subpopulations of the detailed model. The detail model indicates that the influx of thymocyte progenitors is quite small, which agrees well with other estimates.

In addition, we observe that the recovery of DP thymocytes is much faster than that of DN thymocytes. Our model predicts that DP thymocytes accelerate their recovery of population size by proliferating temporally upon the decrease in its population size. A subsequent experiment of proliferation assay verifies this prediction by showing that the proportion of proliferating DP thymocytes gets higher temporarily after irradiation. We also demonstrate that the model of SP4 thymocytes and mTECs can mimics the previous study which impaired interaction between them.

Our model establishes a pivotal step towards the integrative understanding of T cell development as a regulatory network system. We anticipate a future extension of our model by incorporating the dynamics of other thymic resident cells, such as B cells, dendritic cells, and thymic endothelial cells, to understand thymic development and its homeostasis more comprehensively.

Although the mathematical model for population dynamics in the thymus revealed the contribution of intercellular interactions to homeostasis of thymic populations size, it remains unclear how the TCR repertoire diversity, the quality of T cell population, is maintained against perturbation. Therefore, we next investigate the dynamics of the TCR repertoire change by thymic selection in which thymocytes with inappropriate TCRs are weeded out via interactions with TECs. We perform deep sequencing of TCR α and β chains from matured DP and SP4 thymocytes during the recovery from irradiation to measure the effect of negative selection, an elimination of self-reactive TCRs, and its temporal disorder by irradiation. TCR repertoire analysis clarifies that the repertoires of α and β chains are temporally impaired by irradiation in different ways.

In the normal TCR α chain repertoire before irradiation, we find that the unique α chain derived from invariant natural killer T cells (iNKT cells) becomes significantly abundant in SP4 thymocytes. However, irradiation curtailed the abundance of the unique α chain. We also observe that the recovery of the unique α chain abundance is much slower than that of the other α chains. This slower recovery suggests that the iNKT-like fraction undergoes different differentiation process from other thymic T cells.

We also characterize the difference of TCR β chain repertoire by the usage of V and J genes, which code variable regions of TCR chains. We find that linear transformations of V and J gene usage counts quantify the effect of negative selection and irradiation. Furthermore, we also find that the effect of both negative selection and irradiation are correlated more with V genes than with J genes. This correlation of the irradiation effect with V genes suggests that the TCR, especially its V gene, may contribute to the thymocyte ability to survive and proliferate after irradiation. We next investigate how the proportion of common CDR3 sequences, one of the variable regions of the TCR, changes after irradiation. We find that the proportion of several CDR3s gets higher after irradiation. This also supports the prediction of TCR-dependent radiation tolerance, because thymocytes of small population size under normal conditions would not get abundant by irradiation if every clone is equally eliminated by irradiation.

These results can serve as a first step of the forthcoming study of the repertoire time course to dissect the mechanism of thymic selection and the repertoire homeostasis in the thymus.

Finally, we investigated developmental control of thymocytes phenotypes. The phenotype composition of T cells, as well as their TCR repertoire diversity, is also vital for appropriate immune reaction. The lineage choice of thymocytes in the thymus is the first step that shapes various phenotypes of T cells. Despite the exploration of numerous molecules that engage in the lineage choice, it remains unclear how those molecules as a whole process multiple extracellular signals for appropriate differentiation.

To address this problem, we construct a mathematical model of intracellular signaling during thymocyte differentiation. In particular, we focus on a homeostatic property of differentiation duration. The mathematical model suggests that incoherent regulations of the TCR signal to the cytokine signal contributes to the constant differentiation duration independently from TCRs. We expect that our model evokes experimental verification of the model.

Our quantitative studies exploit mathematical modeling and sequence analysis, emerging approaches in immunology, to provide a systematic viewpoint that integrates previous findings on various aspects of T cell development, and deepen understanding of the homeostasis in the thymus. We anticipate that our studies have a pivotal impact on future directions for immunological research from a systematic perspective.