



Impaired lymphocyte trafficking in mice deficient in the kinase activity of PKN1

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(課程博士関係)

学位論文の内容要旨

Impaired lymphocyte trafficking in mice deficient in the kinase activity of PKN1

PKN1 キナーゼ活性欠失マウスにおけるリンパ球のトラフ ィッキング障害について

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Summary

Protein kinase N (PKN) is a serine/threonine protein kinase with a catalytic domain homologous to that of protein kinase C (PKC) and a unique regulatory region containing antiparallel coiled-coil (ACC) domains. PKN1, also known as PKN α or PRK1, is one of three PKN isoforms derived from different genes in mammals. PKN1 was first described as a fatty acid- and phospholipid-activated serine/threonine protein kinase and a protease-activated protein kinase. PKN1 is also an effector protein kinase of Rho family GTPases, such as RhoA, RhoB, RhoC, and Rac, in mammalian tissues. Various PKN1 functions have been revealed using cell culture experiments; for example, it is involved in the regulation of cytoskeletal reorganization, cell adhesion, cell-cycle regulation, and tumorigenesis. PKN1 functions as an intracellular signalling molecule, in some cases independent of phosphorylation activity, e.g., it activates phospholipase D1 and acts as a scaffold protein for the p38 γ MAPK signalling pathway. PKN1 knockout (KO) mice appear normal and do not exhibit defects in lymphocyte development in PKN1 KO mice within 12 weeks of age. Germinal centers form spontaneously in the spleen at more than 30 weeks of age in PKN1 KO mice, even in the absence of immunization or infection, and these mice eventually develop an autoimmune-like disease characterized by autoantibody production and glomerulonephritis. Newly generated lymphocytes migrate from the bone marrow or thymus into the blood and travel to secondary lymphoid organs, such as the spleen and lymph nodes. Entry from the blood to lymphoid tissues involves a multistep cascade, including selectin-mediated cell rolling, followed by chemokine-triggered integrin activation, integrin-mediated adhesion, and transmigration across the endothelium. After surveying the secondary lymphoid organs, T and B cells egress to the blood and lymph, migrating to other lymphoid organs and continuing surveillance. Major driving force that mediates lymphocyte egress from lymphoid organs is the concentration differential of S1P between luminal and abluminal compartments via stimulation of the lymphocyte S1P receptor. The lipid ligand S1P is ubiquitously synthesized, but is largely degraded in most tissues, resulting in low ligand levels in lymphoid tissues and higher concentrations in the blood and lymph. The S1P receptor 1 (S1PR1), one of the five G protein-coupled S1P receptors, has been shown to control lymphocyte egress from the thymus, spleen, and lymph nodes, based on S1PR1 deletion experiments. Therefore, to explore the role of the phosphorylation activity of PKN1 in vivo, we generated knock-in mice expressing kinase-negative mutant of PKN1 by introducing a T778A point mutation in the activation loop of the catalytic domain. PKN1[T778A] mutant mice developed to adulthood without apparent external abnormalities, but exhibited lower T and B lymphocyte counts in the peripheral blood than those of wild-type (WT) mice. T and B cell development proceeded in an apparently normal fashion in bone marrow and thymus of PKN1[T778A] mice, however, the number of T and B cell counts were significantly higher in the lymph nodes and spleen of mutant mice in those of WT mice. After transfusion into WT recipients, EGFP-labelled PKN1[T778A] donor lymphocytes were significantly less abundant in the peripheral circulation and more abundant in the spleen and lymph nodes of recipient mice compared with EGFP-labelled WT donor lymphocytes, likely reflecting lymphocyte sequestration in the spleen and lymph nodes in a cell-autonomous fashion. PKN1[T778A] lymphocytes showed significantly lower chemotaxis towards chemokines and sphingosine 1-phosphate (S1P) than WT cells in vitro. The

biggest migration defect was observed in response to S1P, which is essential for lymphocyte egress from secondary lymphoid organs. PKN1[T778A] lymphocytes accumulated in the spleen as well as in lymph nodes. Analyses of S1PR1-deficient fetal liver chimeras and T cell-specific S1PR1 knockout mice have revealed peripheral lymphocyte deficiencies and the thymic accumulation of mature CD4 and CD8 single-positive T cells at the same time. This mature thymocyte accumulation has also been observed in mice treated with antagonist of S1P, FTY720. However, PKN1[T778A] mice did not exhibit the accumulation of CD4 and CD8 single-positive T cells in the thymus. The cell-surface expression level of S1PR1 did not differ significantly between [T778A] and WT lymphocytes; therefore, the PKN1[T778A] mutation seems to impair a signalling step downstream of the S1P receptor in lymphocytes. PKN1 has important roles in cell migration and invasion in various adherent cell lines, including prostate adenocarcinoma cell lines, such as PC-3 and LNCaP stimulated by thromboxane and androgen, androgen-independent prostate tumour cell lines, such as PC-3M-luc2 and Du145, the triple-negative breast cancer cell line MDA-MB-231, the bladder tumour cell line 5637, and human aortic smooth muscle cells stimulated by monocyte chemoattractant protein (MCP)-1. In these cases, PKN1 has been suggested to be involved in processes downstream of Rho or Rac GTPases, major molecular switches of cell migration and cytoskeletal regulation in these cells. Rho and Rac GTPases are also reported to play major roles in lymphocyte migration as follows. i) RhoA and Rac1 influence chemokine-induced T-cell polarity, which is crucial for *in vivo* migration. ii) Chemokines, such as CCL21, CXCL13, and CXCL12, bind to G-protein-coupled receptors, leading to the activation of Rho and Rac GTPases in B cells. iii) S1P binds to G-protein-coupled S1PR1, which activates the RhoA-specific guanine nucleotide exchange factor (GEF) Lsc (also known as ARHGEF1) and the Rac-specific GEF DOCK2, leading to the migration and egress of lymphocytes from lymph nodes. In our study, the migration activity of PKN1[T778A] lymphocytes was lower than that of WT cells when stimulated by chemokines (CCL21 for T cells, CXCL13 for B cells, and CXCL12 for T and B cells. Therefore, it is likely that PKN1 is widely involved in chemokine- and S1P-induced lymphocyte migration downstream of Rho or Rac GTPase functions. PKN1[T778A] mice showed a characteristic set of trafficking abnormalities, i.e. deficiencies in both T and B cells in the peripheral blood, the accumulation of T and B cells in lymph nodes and the spleen, a lack of changes in primary lymphoid organs, and normal numbers of other hematopoietic cells, such as eosinophils, basophils, platelets, and red blood cells. The same set of phenotypes has not been observed in other genetically modified mice to date. These results reveal a novel role of PKN1 in lymphocyte migration and localization.

論文審査の結果の要旨			
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論文題目 Title of Dissertation	PKN1 キナーゼ活性欠失マウスにおけるリンパ球のトラフィック グ障害について Impaired lymphocyte trafficking in mice deficient in the kinase activity of PKN1		
審査委員 Examiner	主 査 田村 裕雄 Chief Examiner 副 査 平島 正則 Vice-examiner 副 査 杉本 幸司 Vice-examiner		

PKN1 was first described as a fatty acid- and phospholipid-activated serine/threonine protein kinase and a protease-activated protein kinase. The candidate generated knock-in mice expressing kinase-negative mutant of PKN1 by introducing a T778A point mutation in the activation loop of the catalytic domain. PKN1[T778A] mutant mice developed to adulthood without apparent external abnormalities, but exhibited lower T and B lymphocyte counts in the peripheral blood than those of wild-type (WT) mice. T and B cell development proceeded in an apparently normal fashion in bone marrow and thymus of the mice, however, the number of T and B cell counts were significantly higher in the lymph nodes and spleen of mutant mice in those of WT mice. After transfusion into WT recipients, EGFP-labelled PKN1[T778A] donor lymphocytes were significantly less abundant in the peripheral circulation and more abundant in the spleen and lymph nodes of recipient mice compared with EGFP-labelled WT donor lymphocytes, likely reflecting lymphocyte sequestration in the spleen and lymph nodes in a cell-autonomous fashion. PKN1[T778A] lymphocytes showed significantly lower chemotaxis towards chemokines and sphingosine 1-phosphate (S1P) than WT cells in vitro. The biggest migration defect was observed in response to S1P, which is essential for lymphocyte egress from secondary lymphoid organs. PKN1[T778A] lymphocytes accumulated in the spleen as well as in lymph nodes. The mice did not exhibit the accumulation of CD4 and CD8 single-positive T cells in the thymus. The cell-surface expression level of S1PR1 did not differ significantly between [T778A] and WT lymphocytes,

therefore, the PKN1[T778A] mutation seems to impair a signaling step downstream of the S1P receptor in lymphocytes. It is likely that PKN1 is widely involved in chemokine- and S1P-induced lymphocyte migration downstream of Rho or Rac GTPase functions. PKN1[T778A] mice showed a characteristic set of trafficking abnormalities, i.e. deficiencies in both T and B cells in the peripheral blood, the accumulation of T and B cells in lymph nodes and the spleen, a lack of changes in primary lymphoid organs, and normal numbers of other hematopoietic cells, such as eosinophils, basophils, platelets, and red blood cells. The same set of phenotypes has not been observed in other genetically modified mice to date. These results reveal a novel role of PKN1 in lymphocyte migration and localization.

The candidate completed the studies on mice lymphocytic trafficking in deficient in the kinase activity of PKN1, and having advanced the field of knowledge in the area of medicine, we hereby recognized the candidate as having qualified for the degree of Ph. D. (Medicine).