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学 位 論 文 題 名

Effect of glycogen synthase kinase 3 activity on dendritic cell function to induce T helper 2 polarization

(樹状細胞の Th2細胞誘導能と glycogen synthase kinase 3)

学位論文内容の要旨

Introduction

Dendritic cells (DC), the most potent antigen-presenting cells, play a critical role in both innate and adaptive immunity by producing chemokines and cytokines as well as presenting antigens to the antigen-specific T cells. DC polarize naive $CD4^+$ T cells to T helper 1 (Th1) or Th2 cells. Th1 cells, producing interferon (IFN) -γ, are crucial in promoting cellular immunity that clear intracellular bacteria and viruses, whereas Th2 cells, releasing interleukin (IL)-4, IL-5, IL-10, and IL-13, induce humoral immune responses against extracellular parasites. However, the mechanism underlying the DC regulation of Th1 or Th2 differentiation is not fully understood. Glycogen synthase kinase 3 (GSK3), a serine/threonine kinase, is involved in the Wnt signaling pathway. GSK3 regulates many cellular functions including glycogen metabolism, cell-cycle control, and proliferation. GSK3 can both positively and negatively affect a variety of transcription factors that are critical in regulating pro- and anti-inflammatory cytokine synthesis. GSK3 is a crucial enzyme for the differentiation and maintenance of an immature phenotype of human monocyte-derived DC. However, a role of GSK3 in the DC function for Th1 or Th2 differentiation remains unclear. In the present study, the role of GSK3 activity during the development of DC from murine bone marrow (BM) cells to functional DC was examined.

Materials and methods

DC were generated by culturing lineage-marker-negative BM cells of C57BL/6 mice with granulocytes-macrophage colony-stimulating factor for 6 days in the presence or absence of a specific inhibitor of GSK3, SB415286, and used as GiDC or control DC. To examine the inhibitory effects of SB415286 on GSK3 activity in murine BM cells, the level of phosphorylated glycogen synthase in B6 BM cells lysates treated with or without SB415286 were evaluated by immunoblotting. Expression of cell surface molecules on each type of DC was analyzed by flow cytometry. To evaluate the ability to induce Th1 or Th2 differentiation, control DC or GiDC were mixed with allogeneic (allo)-$CD4^+$ T cells of BALB/c mice for 5 days. To determine cell proliferation activity, after the coculture with each type of DC for 68 hours, the cells were pulsed with [^{3}H] thymidine for 4 hours and then harvested onto glass fiber. Incorporation of [^{3}H] thymidine was measured with a liquid scintillation counter. In addition, the $CD4^+$ T cells cultured with each type of DC were restimulated with anti-CD3 monoclonal antibody (mAb) for 24 hours. To assess CD40-mediated DC function, control DC or GiDC were stimulated with anti-CD40 mAb for 24 hours. To examine *in vivo* DC function, control DC or GiDC from B6 mice were injected into

BALB/c mice via the tail vein and five days after the injection, splenic $CD4^+$ T cells were cultured on anti-CD3 mAb coated well for 24 hours. To compare the ability for cytokine production in response to lipopolysaccharide (LPS) between control DC and GiDC, these DC were stimulated with LPS for 24 hours. The protein levels of cytokines (IL-4, IL-6, IL-10, IL-12 p40 and IFN-γ) in the culture supernatants were evaluated by enzyme-linked immunosorbent assay (ELISA).

Results

Treatment of the BM cells with SB415286 significantly decreased the spontaneous phosphorylation of glycogen synthase. Thus, SB415286 inhibited GSK3 activity in BM cells. Either GiDC or control DC exhibited a conventional DC phenotype ($CD11b^+$ $B220^-$ $CD8^-$). CD40, CD80, CD86, and $I\text{-}A^b$ expression on GiDC was significantly decreased as compared to that on control DC. The proliferation activity of allo-$CD4^+$ T cells stimulated with GiDC was lower than that of cells stimulated with control DC. Level of IFN-γ production in the culture of allo-$CD4^+$ T cells with GiDC was significantly higher than that with control DC. On the contrary, IL-4 and IL-10 production in the culture with GiDC were markedly reduced as compared to that with control DC. Upon restimulation with anti-CD3 mAb, $CD4^+$ T cells primed with GiDC showed decreased IL-4 and IL-10 production and enhanced IFN-γ production compared to those primed with control DC. In addition, ability of GiDC to induce Th2 response *in vivo* was significantly lower than that of control DC. CD40 ligation induced IL-6 and IL-12p40 production by control DC, but failed to induce these cytokine production by GiDC. Levels of IL-12p40 and IL-6 in the culture of allo $CD4^+$ T cell with GiDC were significantly lower than those with control DC. The LPS-induced productions of IL-6 and IL-10 by GiDC were more vigorous than those by control DC. In contrast, LPS-induced IL-12p40 production by GiDC was significantly reduced cpmpared to that by control DC.

Discussion

DC induce polarization of naive $CD4^+$ T cell to Th1 or Th2 effector cells. In particular, the DC-derived factors responsible for the initiation of Th2 differentiation are poorly characterized compared to those of Th1 differentiation. The present findings demonstrate that DC developed under conditions in which GSK3 activity was significantly inhibited (GiDC) showed severely impaired ability to induce Th2 polarization in responding $CD4^+$ T cell in an allo-MLR system *in vitro* and *in vivo*. On the other hand, the GiDC capability to induce Th1 differentiation was sustained or rather augmented. Thus, GSK3 appeared to play a pivotal role in DC development of the capability to induce Th2 polarization in the allo-MLR system. CD28 mediated signals are induced by interaction with the ligands, CD80 and CD86, expressed on DC during the T cell priming. GiDC was almost negative for CD86, whereas CD80 expression was substantially sustained on the GiDC compared to that on control DC. It has been reported that CD28 signals via interaction with CD86 preferentially promote Th2 differentiation compared with those via interaction with CD80. Thus, the marked deficiency of CD86, but not CD80, expression on GiDC may be associated with the impaired function to induce Th2 polarization. The impaired IL-6 production appeared to be attributable to the deficiency of CD40 expression on GiDC. Furthermore, IL-6 level in the culture of allo-MLR with GiDC was significantly reduced compared with that with control DC. From these observations, the impaired ability of GiDC to promote Th2 differentiation might be attributable to their reduced CD40 expression and the low production of IL-6 during the antigen presentation, in addition to the altered interaction with the $CD4^+$ T cells via CD28. The precise mechanism underlying the impaired function of GiDC for Th2 polarization remains unclear and should be persued in further studies. And more, GSK3 activity during the generation of DC appears to be involved in the regulation of the DC ability to induce cytokine production in response to LPS. The present findings may lead to the

development of clinical applications exploiting the new regulation system of DC via GSK3 for the treatment of transplantation, cancer, and various immune disorders.

学位論文審査の要旨

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Effect of glycogen synthase kinase 3 activity on dendritic cell function to induce T helper 2 polarization

(樹状細胞のTh2細胞誘導能とglycogen synthase kinase 3)

樹状細胞（DC）は生体内でもっとも強力な抗原提示細胞で、自然免疫、獲得免疫の両方において重要な役割を果たしている。また、Glycogen synthase kinase 3 (GSK3)は糖代謝、細胞周期、細胞増殖といった多くの細胞機能を制御することが知られている。本研究は、DCを分化誘導してくる際のGSK3活性の役割を検討したものである。DCをGSK3活性抑制下に誘導したDC（GiDC）は、機能的により未熟で、LPSによるIL-12p40産生が減弱していた。Allo-MLRを用いた検討では、GiDCはCD4$^+$T細胞のTh2への分化を有意に抑制し、Th1への分化を誘導していた。また、in vivoの系でも、GiDCはCD4$^+$T細胞のTh2への分化を有意に抑制していた。本研究により、DC分化時におけるGSK3活性が、allo-MLRの系においてDCのCD4$^+$T細胞の分化誘導、とくにTh2への分化の確立に必要なものであることが示された。

質疑応答において、笠原正典教授より、GSK3には2つのisozymeが存在するが、マウス細胞においてどちらも発現するのか、今回用いたinhibitorはどちらも抑制するのか、DCで重要なものはどちらかについての質問があった。この質問に対し、マウスを含め哺乳類では機能の相補性はないものの、2つのisozymeがともに存在していること。今回用いたinhibitorのSB415286は2つのisozymeをともに抑制すること。そして、DCにおいてどちらのisozymeが重要かは現在までのところ検証されていないが、GSK3βのknockoutマウスではTNFαが強発現することで肝細胞のapoptosisが誘導され、重度の肝変性により胎生致死に至ることが報告されており、GSK3が細胞形成においてcriticalな酵素であると回答した。また、GSK3は糖産生制御酵素だが他の基質への影響についての質問があった。この質問に対し、糖代謝のみならず、Wnt/β-cateninに代表される様々なpathwayにおいてもリン酸化をおこすと回答した。その中で、GiDCのTh2分化抑制の機序として、Wnt/β-cateninが特に関与するか質問されたが、本研究では検討しておらず、今後検討していきたいと述べた。また、DCに関してGSK3を着目した経緯についての質問があった。この質問に対し、Human monocyteでのTLR刺激によるIL-10産生上昇のメカニズムが報告されており、移植でのtolerance誘導にIL-10を強力に産生するDCを誘導できないか考えた。DCでもGSK3活性抑制によりIL-10の産生上昇は認めたがtoleranceを誘導しうるものではなかったと回答した。

ついで，小野江和則教授より、DC-$CD4^+$ T 細胞間での抗原提示量が少ない場合に Th2 への偏向が誘導される報告があるが、この検討を行っているかについての質問があった。この質問に対し、allo-MLR における DC、$CD4^+$ T 細胞の比をふって検討したが、アロ抗原提示量は GiDC での Th2 分化抑制に影響を与えなかったと回答した。また、ultrapure LPS を用いた場合の GiDC の IL-10 産生ついての質問があった。この質問に対し、TLR2 ligand 成分を除去してある ultrapure LPS の使用による IL-10 産生量への影響は、今後の検討課題であると回答した。さらに、野々村克也教授より、inhibitor の臨床応用についての質問があった。この質問に対し、SB415286 のヒトへの使用例はないが、oncogene であるβ-catenin の上昇とそれによる発癌性が危惧されるので、axin との重合に関与しない GSK3 inhibitor の開発が望まれると回答した。また、間質性膀胱炎における具体的な投与方法を含めての臨床応用についての質問があった。この質問に対し、間質性膀胱炎は systemic なアレルギー疾患と捉えられるものであり、Th2 抑制目的に GSK3 活性を介する治療は検討してもよいが、局所投与における治療効果は不明であり、全身投与としては Alzheimer 病で使用されている間接的 GSK3 活性抑制能を有するリチウムを用いる方法が考えられると回答した。

この論文は、Immunology で高く評価され、今後の GSK3 活性を介する新たな DC の制御機構の開発に結びつくものと期待される。

審査員一同は、これらの成果を高く評価し、大学院課程における研鑽や取得単位なども併せ申請者が博士（医学）の学位を受けるのに十分な資格を有するものと判定した。