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学位論文名	IL-6 Regulates In Vivo Dendritic Cell Differentiation through STAT3 Activation （IL-6は生体内にて樹状細胞の分化をSTAT3依存性に制御する）
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論文内容の要旨

〔目的〕

It has been reported that IL-10 induces an inhibitory signal for DC maturation in vitro and that both IL-6 and IL-10 trigger the activation of STAT3 molecules. Our hypothesis is that IL-6 regulates DC differentiation in vivo.

〔方法ならびに成績〕

To investigate the effect of IL-6 on DC differentiation in vivo, we analyzed the number of resting/immature and activated/mature DCs in superficial lymph nodes by observing the expression of MHC class II (class II) and CD86 on $CD11c^+$ cells in different mouse strains. We analyzed the $CD11c^+$ DCs in lymph nodes from mice deficient in IL-6 (IL-6KO) and others that had defective gp130-mediated signaling via SHP2 ($gp130^{F759/F759}$) or STAT3 ($gp130^{FxxQ/FxxQ}$), and mice that had both defective gp130-mediated SHP2 signaling and a lack of IL-6 ($gp130^{F759/F759}$/IL-6KO). We observed an enhanced expression of class II and CD86 in the DCs from IL-6KO mice compared with the control C57BL/6 mice, indicating that IL-6 normally suppressed the activation/maturation of DCs in vivo. Interestingly, the numbers of activated/mature DCs that expressed high levels of class II and CD86 decreased in the $gp130^{F759/F759}$ mice, which have enhanced gp130-mediated STAT3 activation but not gp130-mediated SHP2 activation. In addition, DCs from reconstituted $gp130^{FxxQ/FxxQ}$ mice, in which gp130-mediated STAT3 activation is defective, had more activated/mature DCs than reconstituted control mice did, and they had a similar phenotype to the DCs from IL-6KO mice. The suppression of class II and CD86 expression on DCs was recovered in the progeny of $gp130^{F759/F759}$ mice crossed with IL-6KO mice. These results further strengthen the idea that IL-6 plays a suppressive role in vivo. Moreover, they suggest that IL-6 suppresses DC activation/maturation through STAT3 activation in vivo. In contrast to IL-6KO mice, the DCs from IL-10KO mice unexpectedly had almost the same phenotype as the control mice.

To investigate whether IL-6 acts directly on DCs rather than indirectly via another cell population in vivo, a

pure population of DCs was prepared from bone marrow (BMDCs). We stimulated the BMDCs with IL-6 alone, LPS alone, or IL-6 plus LPS, and analyzed the expression of class II, CD86, and CD80. The Class II, CD86, and CD80 expression levels were strongly enhanced by LPS treatment, but suppressed by pretreatment with IL-6. To investigate the importance of STAT3 activation for the IL-6-mediated inhibition of DC maturation/activation, BMDCs were prepared from $gp130^{F759/F759}$ and $gp130^{FxxQ/FxxQ}$ mutants and control animals. All the BMDCs tested showed increased expression of class II and CD86, after LPS stimulation. However, pretreatment with IL-6 suppressed the LPS-mediated up-regulation of class II molecules in the BMDCs from both $gp130^{F759/F759}$ and control mice. The inhibitory effect of IL-6 was greater for the BMDCs from $gp130^{F759/F759}$ mice than for those from control mice. In contrast, IL-6 had no inhibitory effect on the BMDCs from $gp130^{FxxQ/FxxQ}$ mice. These results suggested that the STAT3-but not the SHP-2/Gab/MAPK-dependent pathway is critical for the IL-6-mediated suppression. To confirm the direct involvement of STAT3 in the IL-6-mediated signaling for BMDC maturation/activation, we infected $gp130^{F759/F759}$ BMDCs with a retrovirus vector carrying a dominant-negative STAT3 (DN-STAT3). DN-STAT3 suppressed the IL-6-mediated inhibition of DC activation/maturation compared with control vector-infected BMDCs. Together, these results clearly demonstrate that STAT3-mediated signaling through gp130 is crucial for the IL-6-dependent suppression of DC activation/maturation. To examine the functional significance of the IL-6 effect on DC differentiation, we tested the ability of DCs to present antigen to T cells in vivo. We prepared $CD8^+$ T cells from C57BL/6/OT1/Thy1.1 transgenic mice (see Methods), whose TCR recognizes the OVA peptide on D^b class I molecules. To exclude direct presentation of the OVA epitope on the D^b class I molecules of dying cells, but to see the indirect presentation of the epitope on the $CD8^+$ $CD11c^+$ DCs of the mutant mice after endocytosing the dying cells, we prepared OVA-treated dying cells from the D^bK^b knockout and iv-injected them into IL-6KO, $gp130^{F759/F759}$, and control mice one day after transferring the $Thy1.1^+$ OT1 T cells (day 0). The actual number of OT1 T cells was calculated for days 2, 4, and 7. The number of OT1 T cells was higher in the IL-6KO but lower in the $gp130^{F759/F759}$ mice, compared with control animals, especially on day 4. The differences for each animal group were significant by Student's t-test on day 4 ($P<0.05$).

〔総括〕

Our results show IL-6 to be a major cytokine involved in the control of DC differentiation in vivo, at least in affecting the transition from the resting/immature phenotype to the activated/mature phenotype, in the steady state. It may be more important that this modification of DC differentiation altered the magnitude of the T-cell responses. Increases in IL-6-induced signaling seem to induce decreases in the activation of T cells, suggesting that IL-6 acts as an immunosuppressive cytokine for DC differentiation.

論文審査の結果の要旨

本論文においては、獲得免疫反応の主役であるT細胞を活性化する主要な抗原提示細胞として樹状細胞が挙げられるが、その成熟度をIL-6が制御していることを発見した。

具体的にはIL-6欠損マウスの生体内において樹状細胞の成熟度が亢進しており、さらに本研究室において樹立された gp130 の点変異マウスの生体内において樹状細胞の成熟度が変化していた。これらの結果から gp130 を介したSTAT3のリン酸化が樹状細胞の成熟度の制御に深く関わっていることが示唆された。次に試験管内で培養した樹状細胞にドミナントネガティブのSTAT3を導入する実験を行った。ドミナントネガティブのSTAT3を導入した樹状細胞はその成熟度が上昇しており、これらの結果から樹状細胞の成熟度にSTAT3が抑制的に働くことが示された。

最後にIL-6を介した樹状細胞の成熟度の変化が生体内のT細胞の活性化にも関与していることを示し、生体内の免疫反応におけるIL-6の重要性を示した。

以上の研究は博士（医学）の学位授与に値する。