論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	TEKGUC Murat
論文題名 Title	Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells (制御性T細胞に発現するCTLA-4はトロゴサイトーシスにより抗原提示細胞上のCD80/CD86を除去し遊離PD-L1を発現させる)

論文内容の要旨

〔目　的(Purpose)〕

Foxp3-expressing $CD4^+CD25^+$ regulatory T cells (Tregs) constitutively and highly express the immune checkpoint receptor cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), whose Treg-specific deficiency causes severe systemic autoimmunity. As a key mechanism of Treg-mediated suppression, Treg-expressed CTLA-4 downregulates the expression of CD80/CD86 costimulatory molecules on antigen-presenting cells (APCs). Accumulating evidence also indicates that CD80 not only interacts, in trans, with CD28 and CTLA-4 expressed on T cells but also, in cis, with programmed death ligand-1 (PD-L1) protein expressed by APCs. These cis-CD80/PD-L1 heterodimers on APCs limit the availability of free PD-L1, thereby interfering with the trans-binding between PD-L1 and its receptor PD-1 expressed on T cells. It can be asked then whether Treg-expressed CTLA-4 is able to control the availability of PD-L1 through modulating CD80 expression on APCs. Therefore, it needs to be determined how CTLA-4 precisely modifies the expression profile of APCs for the suppression of conventional T cells (Tconvs).

〔方法ならびに成績(Methods/Results)〕

Methods: In order to determine the role of the cytoplasmic tail portion of CTLA-4 in its cell-extrinsic function, we used tailless (TL) CTLA-4 transgenic (TLC4Tg) mice, whose T cells expressed a mutant CTLA-4 protein without its cytoplasmic tail portion. For functional comparison with TL Tregs, CTLA-4 knockout (KO) and wild-type (WT) CTLA-4-expressing Tregs were isolated from female $CTLA\text{-}4^{flox/flox}$, $Foxp3^{IRES\text{-}Cre}$ heterozygous, Rosa-RFP-reporter mice, which harbored both WT and KO Tregs without developing spontaneous autoimmunity. For tracking the CD80/CD86 molecules expressed on the dendritic cell (DC) surface following their interaction with Tregs, we generated three types of gene-transduced murine DC lines by using CD80-GFP or CD86-GFP fusion protein or GFP alone. Moreover, to examine whether CTLA-4-driven uptake of APC membrane proteins by Tregs might also occur *in vivo*, purified WT or KO Tregs from CD45.2 CRF mice, together with Tconvs from CD45.1 congenic mice, were transferred into syngeneic CD45.1 RAG2 KO mice for assessing the capture of CD45.1 protein by the transferred $CD45.2^+$ WT or KO Tregs. Meanwhile, in order to investigate the effect of CTLA-4-dependent trogocytosis on cis-CD80/PD-L1 heterodimers expressed by DCs, we cocultured these Tregs with freshly purified murine splenic DCs. For analysis, we labeled DCs first with 1-111A anti-PD-L1 monoclonal antibody (mAb), which was previously reported to detect total (CD80-bound and free) PD-L1 molecules, and then with 10F.9G2 PD-L1 mAb, which competes with CD80 for binding and stains free PD-L1.

Results: Here we showed that Treg-expressed CTLA-4 facilitated Treg-APC conjugation and immune synapse formation. The immune synapses thus formed provided a stable platform whereby Tregs were able to deplete CD80/CD86 molecules on APCs by extracting them via CTLA-4-dependent trogocytosis. The depletion occurred even with Tregs solely expressing a mutant CTLA-4 form lacking the cytoplasmic portion required for its endocytosis. Thus, the tail portion of CTLA-4 is dispensable for CD80/CD86 downregulation on APCs and Treg suppressive function. The CTLA-4-dependent trogocytosis of CD80/CD86 also accelerated *in vitro* and *in vivo* passive transfer of other membrane proteins and lipid molecules from APCs to Tregs without their significant reduction on the APC surface. Furthermore, CD80 downregulation or blockade by Treg-expressed membrane CTLA-4 or soluble CTLA-4-immunoglobulin (CTLA-4-Ig), respectively, disrupted cis-CD80/ PD-L1 heterodimers and increased free PD-L1 on dendritic cells (DCs), expanding a phenotypically distinct population of $CD80^{lo}$ free $PD\text{-}L1^{hi}$ DCs.

〔総　括(Conclusion)〕

Tregs are able to inhibit the T-cell stimulatory activity of APCs by reducing their CD80/CD86 expression via CTLA-4-dependent trogocytosis. This CD80/CD86 reduction on APCs is able to exert dual suppressive effects on T-cell immune responses by limiting CD80/CD86 costimulation to naïve T cells and by increasing free PD-L1 available for the inhibition of programmed death-1 (PD-1)-expressing effector T cells. Blockade of CTLA-4 and PD-1/PD-L1 in combination may therefore synergistically hinder Treg-mediated immune suppression, thereby effectively enhancing immune responses including tumor immunity.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

免疫自己寛容において中心的な役割を担う制御性T細胞(Tregs)は、免疫チェックポイント受容体であるCTLA-4を恒常的に高発現しており、CTLA-4を介して抗原提示細胞(APCs)上の共刺激分子CD80/CD86の発現を低下させることで免疫応答を抑制する。本論文では、その詳細な分子機構の解明に取り組み、TregsがCTLA-4依存性のトロゴサイトーシスを介してAPCs上のCD80/CD86を受け取ることでその発現を減少させることを見出した。さらに、TregsはCTLA-4を介したCD80の発現減少に伴い、APCs上のCD80と共抑制分子であるPD-L1のヘテロダイマー形成を阻害し、その結果、APCs上のフリーなPD-L1を増加させることでAPCsによるT細胞の活性化を抑制することも明らかにした。

本論文は、Tregの新たな免疫応答抑制機構に基づく画期的な免疫疾患治療法開発の可能性を提示しており、学位の授与に値すると考えられる。