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学位論文名	A New Subset of CD103＋CD8α＋ Dendritic Cells in the Small Intestine Expresses TLR3, TLR7, and TLR9 and Induces Th1 Response and CTL Activity. （小腸の新規サブセットであるCD103＋CD8α＋樹状細胞は、TLR3, TLR7, TLR9を発現しており、Th1応答と細胞傷害活性を誘導する）
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論文内容の要旨

〔目　的 (Purpose)〕

Our previous report showed that a small number of cells in the lamina propria (LP) could be classified into four subsets based on the difference in CD11c/CD11b expression patterns: $CD11c^{hi}CD11b^{lo}$ DCs, $CD11c^{hi}CD11b^{hi}$ DCs, $CD11c^{int}CD11b^{int}$ macrophages, and $CD11c^{int}CD11b^{hi}$ eosinophils. The $CD11c^{hi}CD11b^{hi}$ DCs, which are $CD103^{+}$, specifically express TLR5 and induce the differentiation of naive B cells into IgA^{+} plasma cells. These DCs also mediate the differentiation of Ag-specific Th17 and Th1 cells in response to flagellin. We found that small intestine $CD103^{+}$ DCs of the LP (LPDCs) could be divided into a small subset of $CD8\alpha^{+}$ cells and a larger subset of $CD8\alpha^{-}$ cells. Flow cytometry analysis revealed that $CD103^{+}CD8\alpha^{+}$ and $CD103^{+}CD8\alpha^{-}$ LPDCs were equivalent to $CD11c^{hi}CD11b^{lo}$ and $CD11c^{hi}CD11b^{hi}$ subsets, respectively. The purpose of this study is to analyze the novel subset of $CD8\alpha^{+}$ LPDCs to elucidate their immunological functions.

〔方法ならびに成績 (Methods/Results)〕

$CD103^{+}CD8a^{+}$ LPDCs were isolated using flow cytometer and analyzed for mRNA expression of TLR family members and RALDH isoforms by RT-PCR and quantitative real-time PCR, respectively. $CD103^{+}CD8a^{+}$ LPDCs were stimulated with TLR ligands and cytokine production was determined by ELISA. $CD103^{+}CD8a^{+}$ LPDCs were co-cultured with peritoneal naive B cells and investigated T-cell independent IgA synthesis. $CD103^{+}CD8a^{+}$ LPDCs were co-cultured with OT-II $CD4^{+}$T cells and analyzed for antigen-specific helper T cell response. In addition, $CD103^{+}CD8a^{+}$ LPDCs were co-cultured with OT-I $CD8^{+}$T cells and antigen-specific CD8 T cell response was investigated. Furthermore, $CD103^{+}CD8a^{+}$ LPDCs were analyzed for antigen-specific cytotoxic T lymphocyte activity. In all experiments, $CD103^{+}$ $CD8a^{-}$ LPDCs were used as a control.

$CD103^{+}CD8\alpha^{+}$ LPDCs expressed TLR3, TLR7, and TLR9 and produced IL-6 and IL-12p40, but not TNF-α, IL-10, or IL-23, following TLR ligand stimulation. $CD103^{+}CD8\alpha^{+}$ LPDCs did not express the gene encoding retinoic acid-converting

enzyme *Raldh2* and were not involved in T cell-independent IgA synthesis or Foxp3$^+$ regulatory T cell induction. Furthermore, CD103$^+$CD8α$^+$ LPDCs induced antigen-specific IgG in serum, a Th1 response, and CTL activity in vivo. Accordingly, CD103$^+$CD8α$^+$ LPDCs exhibit a different function from CD103$^+$CD8α$^-$ LPDCs in active immunity. This is the first analysis, to our knowledge, of CD8α$^+$ DCs in the LP of the small intestine.

〔総　括 (Conclusion)〕

Our data suggest that CD103$^+$CD8a$^+$ and CD103$^+$ CD8a$^-$ LPDCs have divergent functions in active immunity. Based on the results obtained in this study, from both quantitative and qualitative viewpoints, CD103$^+$CD8a$^+$ LPDCs may be less suitable targets for oral vaccines than CD103+CD8a$^-$ LPDCs in the small intestine. Further analysis of these two CD103$^+$ DC subsets as well as their functional cross talk is needed for a better understanding of how the intestinal immunity is regulated to finely tune the innate and adaptive systems.

論文審査の結果の要旨

CD103$^+$ 樹状細胞は、腸管粘膜固有層における主要な抗原提示細胞である。本研究課題で、小腸粘膜固有層のCD103$^+$ 樹状細胞が少数のCD8α$^+$サブセットと多数のCD8α$^-$ サブセットに分かれることを見いだした。フローサイトメトリーを用いた解析から、CD103$^+$CD8α$^+$樹状細胞とCD103$^+$CD8α$^-$樹状細胞はそれぞれCD11c^{hi}CD11b^{lo} とCD11c^{hi}CD11b^{hi}のサブセットに相当することが分かった。２つのCD103$^+$ 樹状細胞サブセットの機能の相違を検討するために、CD103$^+$CD8α$^+$樹状細胞の解析を行った。CD103$^+$CD8α$^+$樹状細胞はTLR3, TLR7, TLR9を発現していたが、CD103$^+$CD8α$^-$樹状細胞はTLR5とTLR9を発現していた。いずれの樹状細胞もTLRリガンドに反応してIL-6やIL-12p40を産生したが、TNF-α, IL-10やIL-23 は誘導しなかった。 CD103$^+$CD8α$^-$樹状細胞だけがレチノイン酸変換酵素RALDH2を発現しており、T細胞依存的なIgA産生やFoxP3$^+$制御性T細胞を誘導した。我々はさらに抗原を貪食させたCD103$^+$CD8α$^+$樹状細胞とCD103$^+$CD8α$^-$樹状細胞を用いて、抗原特異的な免疫応答を検討した。CD103$^+$CD8α$^+$樹状細胞は抗原特異的なIgG産生、Th1応答、細胞傷害性T細胞を誘導した。一方、CD103$^+$CD8α$^-$樹状細胞は抗原特異的なIgGとIgAを、Th1応答だけでなくTh17応答を、そして強い細胞傷害性T細胞活性を誘導した。この様に小腸粘膜固有層の2つのCD103$^+$樹状細胞サブセットが、免疫の活性化において全くことなる機能を有していることを明らかにした。

以上の結果は、学術的に非常に有意義な成果であり、学位の授与に値すると考えられる。