（第３号様式）

学　位　論　文　要　旨

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論　文　名　Ｃキナーゼ阻害剤で誘導されたヒト寛容型樹状細胞の抑制機能の解析―
各種誘導寛容型樹状細胞との比較試験

学位論文要旨

1. Background and purpose

Dendritic cells (DCs) are professional antigen presenting cells involved in initiation of both immunity and immunological tolerance. Tolerogenic DCs (tDCs) are characterized by a semimature phenotype, with a capacity for high antigen uptake and low expression of co-stimulatory molecules.

Most patients with autoimmune diseases and graft transplantation are treated with immunosuppressive drugs that induce generalized immune suppression, thus increasing the risk of infection and cancer. Therefore, to prevent undesirable side effects, the use of antigen specific tDCs that target autoreactive T cells in an attractive strategy, with the aim of reprogramming the immune system.

Human tDCs can be generated ex vivo using various compounds. However, the compounds most suitable for clinical application remain undefined. In the present study, we systematically evaluated seven kinds of tDCs generated ex vivo using the compounds for their tolerogenic potential, stability, and capacity for migration toward secondary lymphoid organ.

2. Material and Methods

1) Agents used in this study were as follows; IL-10; TGF-β1; PPAR γ agonist, 15-deoxy-$\Delta^{-12,14}$ PG J2 (15d-PGJ2) with all trans retinoic acid (ATRA); 1 α dihidroxyvitamin D3 (VitD3); PKCI, bisindolylmaleimide I; dexamethasone (Dexa); and rapamycin (Rapa).

2) Generation of human dendritic cells

After PBMC isolated using $CD14^+$ Isolation Kit, immature DCs (iDCs) were generated from the $CD14^+$ monocytes by culturing them in X-VIVO medium with 2 % human autologous serum, 75 ng/mL rhGM-CSF, and 10 ng/mL rhIL-4 for 5 days. To induce mature DCs (mDCs), iDCs were incubated with a maturation cocktail containing rhTNF-α (10ng/ml), rhIL-1β (10ng/mL) and PGE_2 (1μg/mL) for a further 48 h. Compound-treated tDCs were generated by culturing iDCs with a maturation cocktail in the presence of each compound for 48 h.

3) Other methods : Flow cytometric analysis; in vitro T cell proliferation assay; measurement of cytokine production from DCs; phagocytic ability; induction of regulatory T cells by compound-treated DCs; in vitro T regulatory activity; stability of DCs under proinflammatory stimuli; and chemotaxis assay.

3. Results

1) Comparison of the surface phenotypes of compound-treated human tDCs

We compared the surface expression of costimulatory molecules (CD40, CD80, CD83, and CD86) and MHC Class II on iDCs, mDCs and each of the compound-treated tDCs. The expression levels of costimulatory molecules, CD40, CD80, CD83, and CD86 on tDCs treated with PKCI, IL-10, Vit D3, or PPAR γ+ATRA remained as low as those on iDCs, whereas those on tDCs treated with Dexa, TGF- β, or Rapa were higher than those on iDCs. Upregulation of CCR7 expression was recognized on PKCI-, TGF-β- and Rapa-tDCs. In contrast, IL-10-, Vit D3-, PPAR γ+ATRA-, and Dexa-tDCs showed little or no expression of CCR7 or migration to CCL19.

2) Cytokine production and phagocytic ability of the various compound-treated human tDCs

IL-10 production was high in IL-10-tDCs, moderate in PKCI-tDCs and low in other tDCs, iDCs and mDCs. The production of TGF-β by PKCI-tDCs was higher than by other tDCs, and mDCs.

3) Suppressive properties of the various compound-treated human tDCs

PKCI-IL10-, and Vit D3-tDCs and iDCs reduced the proliferation of allogenic $CD4^+$T cells strongly by 69-86% in comparison with mDCs, whereas Dex-, TGF-β-, and Rapa-tDCs reduced the proliferation weakly by 21-27 %.

4) Induction of functional regulatory T cells by the various compound-treated human tDCs

$CD4^+$T cells co-cultured with PKCI-, or IL-10-tDCs significantly suppressed the proliferation of effector Th1 cells in comparison with those co-cultured with other tDCs.

5) Stability of the tolerogenic properties of the various compound-treated human tDCs

All of the tDCs used in this study were refractory to stimulation with proinflammatory mediators and their tolerogenic properties were stable.

4. Discussion and Conclusion

For clinical application of tDCs, three functional characteristics are required; 1) CCR7-dependent migration toward secondary lymphoid organs, 2) efficient induction of functional regulatory T cells and 3) stability upon exposure to proinflammatory stimuli.

In the present study, we compared seven different protocols for generation of tDCs using compounds suitable for use in clinical trials. PKCI-, IL-10, and VitD3-tDCs appeared to have strong tolerogenic properties and stable phenotypes. However, PKCI-tDCs exhibit CCR7-dependent migration, whereas IL-10-, and Vit D3-tDCs hardly do so. Therefore, PKCI-tDCs may be useful for tolerance-inducing therapy, since they satisfy the required functional characteristics for clinical-grade tDCs.

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