行政院國家科學委員會專題研究計畫　成果報告

CCL21 和 CCR7 對樹突細胞及 T 細胞移行及分化調控之研究

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計畫主持人：吳孟澤

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中英文摘要

Dendritic cells (DC) migrate from peripheral tissues such as skin to regional secondary lymphoid organs via afferent lymphatic vessels, which are known to constitutively secrete the chemokine CCL21. CCL21 and its receptor, CCR7, have been shown to be critical in this pathway. To determine whether lymphatic endothelial cells directly stimulate transendothelial migration of DC, we developed a transendothelial migration assay using murine skin-derived migratory DC (mDC) and cultured ovine lymphatic endothelial cells (LEC) grown on Transwell filters. In standard chemotaxis assays without LEC, mDC migrated 14-fold better to conditioned medium from LEC compared to control medium. This response could be blocked by pretreatment of mDC with CCL21 or pertussis toxin (PTX). While migration in the absence of LEC was minimal, migration was 32-fold better in the presence of a LEC monolayer covering the Transwell filter. While pretreatment of mDC with PTX resulted in a 96% decrease in trans-LEC migration, pretreatment of mDC with CCL21 resulted in a statistically insignificant decrease in migration, suggesting that chemokines other than CCR7 ligand may be responsible for stimulating transendothelial migration. Transendothelial migration was not stimulated by NIH-3T3 cells or murine transformed vascular endothelial cell lines using the same Transwell culture system. Therefore, we postulate that while LEC contribute to both attraction of DC to lymphatic vessels as well as to subsequent transmigration via chemokine-dependent mechanisms, the chemokine receptors involved are not necessarily identical. 以往研究顯示活化之樹突細胞由週邊組織 (如皮膚) 經由週邊淋巴管移行至 secondary lymphoid organs (如淋巴結),這些週邊淋巴管可分泌趨化激素 CCL21. 而 CCL21 與其受體 CCR7 則在這段移行過程中扮演重要角色.為了探討淋巴管內皮細胞是否可以直接刺激樹突細胞直接穿越管壁內皮,我們利用起源於皮膚之樹突細胞進行了標準趨化性測試,並且進一步把羊之淋巴內皮細胞培養於 0.8 μm 之濾膜之上進行穿越內皮細胞之移行測試.在濾膜上缺少淋巴管內皮細胞的標準趨化性測試中,樹突細胞對淋巴管內皮細胞培養液的趨化性比對照組大 14 倍.若先以 CCL21 或是 pertussis toxin 處理樹突細胞則可抑制此反應 (~98% 及~96%).以樹突細胞進一步進行穿越內皮細胞之移行測試,進一步發現樹突細胞穿越有附著內皮細胞的濾膜的程度與對照組比較大了 32 倍.若先以 pertussis toxin 處理樹突細胞可以抑制此反應(~96%), 但是先以 CCL21 處理則無顯著抑制作用,由此推測可能另有其他趨化激素可以促進樹突細胞穿越淋巴管內皮細胞.此外,若以其他細胞(NIH3T3,血管內皮細胞) 附著濾膜進行穿越移行測試,樹突細胞並無法穿越這些細胞.推論由淋巴管內皮細胞分泌之 CCL21 是吸引樹突細胞的主要因子,而淋巴管內皮細胞更可進一步直接促進樹突細胞穿越內皮,由於 pertussis toxin 皆可抑制這些反應,可進一步推論雖然淋巴管內皮細胞對這兩

種反應皆有促進之作用,然而其中所牽涉之趨化激素及其受體則未必完全相同.

報告內容

INTRODUCTION

Activated dendritic cells migrate from peripheral tissues such as skin to regional secondary lymphoid organs (i.e., lymph nodes) via afferent lymphatic vessels, which are known to constitutively secrete the chemokine CCL21. CCL21 and its receptor, CCR7, have been shown to be critical in this migratory pathway. To determine whether lymphatic endothelial cells directly stimulate transendothelial migration of DC, we took advantages of standard in vitro chemotaxis assay and developed a transendothelial migration assay using murine skin-derived migratory DC (mDC) and cultured ovine lymphatic endothelial cells (LEC) grown on Transwell filters.

METHODS AND RESULTS

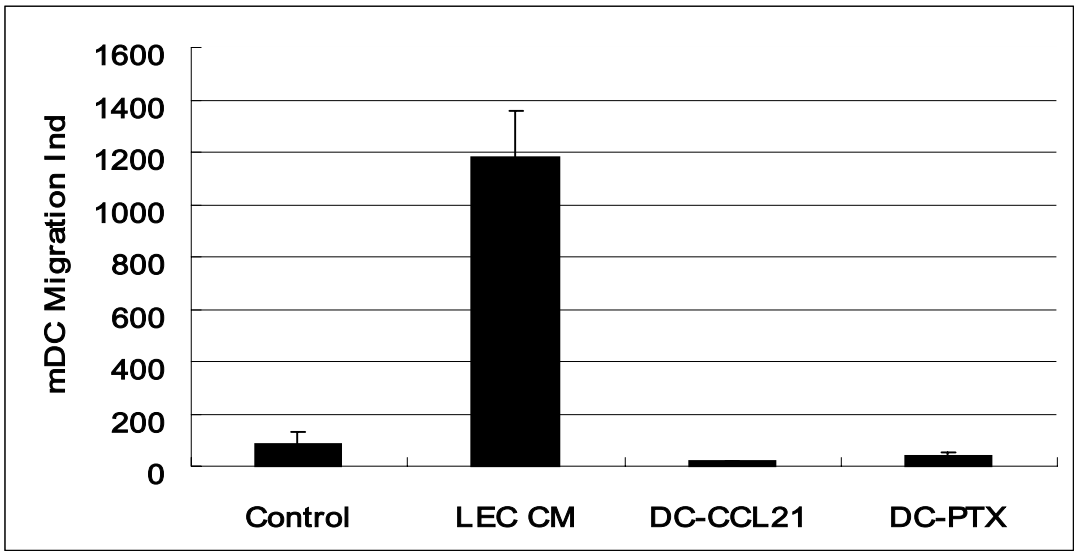


Fig.1 Standard chemotaxis assays. mDC migrated 14-fold better to conditioned medium from LEC (LEC CM) compared to control medium ($p<0.01$). This response could be blocked by pretreatment of mDC with CCL21 (DC-CCL21)(~98% decreas) or pertussis toxin (DC-PTX) (~96% decrease).

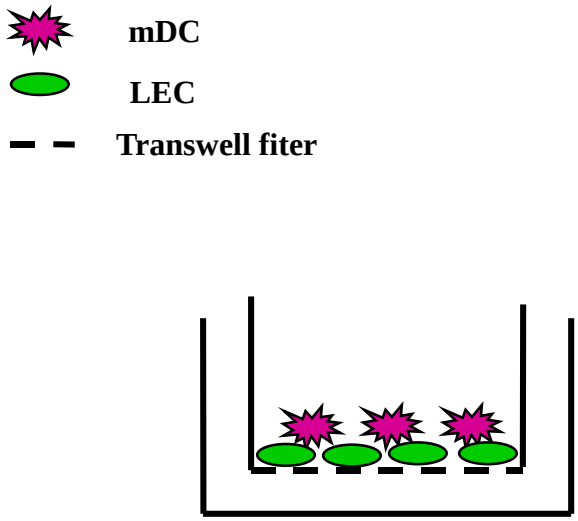
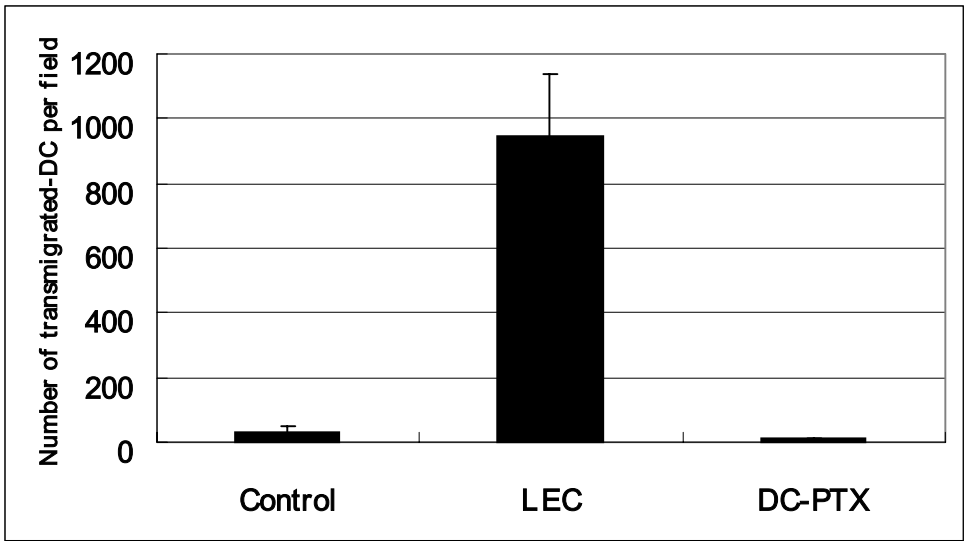


Fig.2 Set-up of transendothelial migration assays. (a) LEC were grown onto Transwell filters and stained with fluorescent before use to confirm the viability of LEC. mDC were then placed on top of the filter and incubated for 3 hrs. Trans-LEC migration of mDC were then measured by counting the mDC cell number in the chamber below transwell filter.



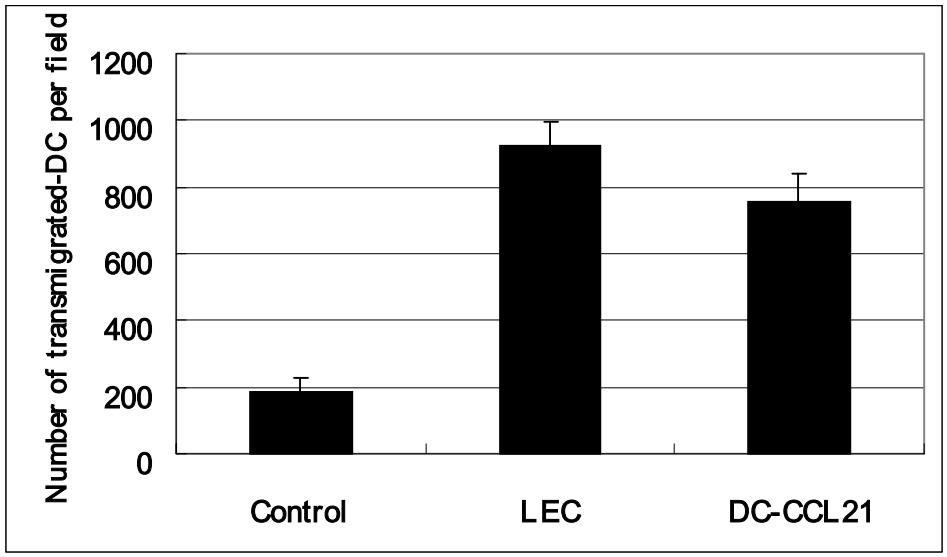
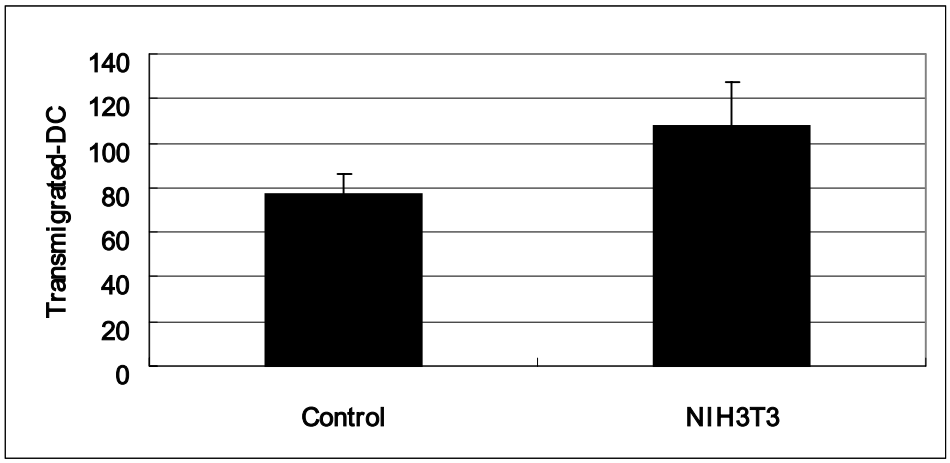


Fig. 3. Results of tranendothelial migration assays. Transwell filters were either covered by LEC as shown in Fig. 2 or not as control. mDC tranendothelial migration were reflexed by number of mDC migrating through filters. (a) While migration in the absence of LEC was minimal, migration was 32-fold better

(p=0.01) in the presence of a LEC monolayer covering the Transwell filter. Furthermore, while pretreatment of mDC with PTX (DC-PTX) resulted in a 96% decrease ($p<0.05$) in trans-LEC migration shown in (a), (b) pretreatment of mDC with CCL21 (DC-CCL21) resulted in a statistically insignificant (p=0.057) decrease in migration.



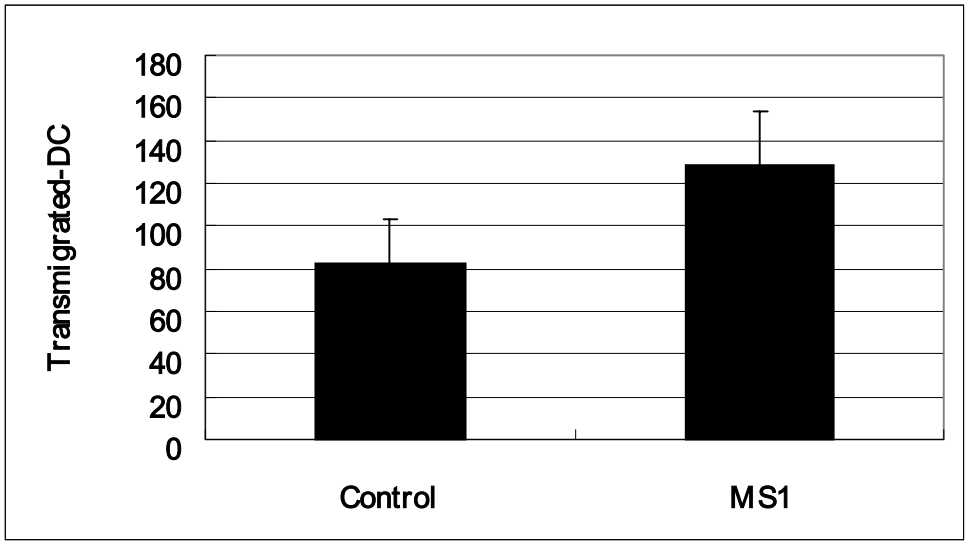


Fig. 4. mDC transendothelial migration was not stimulated by (a) NIH-3T3 cells or murine transformed vascular endothelial cell lines ((b) MS1 and (c) SVR) using the same Transwell culture system.

CONCLUSIONS

1. **CCL21 is a major factor produced by LEC that stimulates chemotaxis of DC.**
2. **LEC, in contrast to vascular endothelial cells, are able to directly stimulate transmigration of DC in a PTX-sensitive fashion.**
3. **While LEC contribute to both attraction of DC to lymphatic vessels as well as to subsequent transmigration via chemokine-dependent mechanisms, the chemokine receptors involved are not necessarily identical.**

References

1. Banchereau, J. and R.M. Steinman, *Dendritic cells and the control of immunity.* Nature, 1998. **392**(6673): p. 245-52.
2. Larsen, C.P., et al., *Migration and maturation of Langerhans cells in skin transplants and explants.* J. Exp. Med., 1990. **172**: p. 1483-93.
3. Lukas, M., et al., *Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model.* J. Invest. Dermatol., 1996. **106**: p. 1293-9.
4. Weinlich, G., et al., *Entry into afferent lymphatics and maturation in situ of migrating murine cutaneous dendritic cells.* J. Invest. Dermatol., 1998. **110**: p. 441-8.
5. Luster, A.D., *Chemokines--chemotactic cytokines that mediate inflammation.* N Engl J Med, 1998. **338**(7): p. 436-45.
6. Baggiolini, M., *Chemokines in pathology and medicine.* J Intern Med, 2001. **250**(2): p. 91-104.
7. Gunn, M.D., et al., *Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization [see comments]*. J Exp Med, 1999. **189**(3): p. 451-60.
8. Gunn, M.D., et al., *A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes.* Proc. Natl. Acad. Sci. (USA), 1998. **95**: p. 258-63.
9. Saeki, H., et al., *Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes.* J Immunol, 1999. **162**(5): p. 2472-5.
10. Dieu, M.C., et al., *Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites.* J Exp Med, 1998. **188**(2): p. 373-86.
11. Yanagihara, S., et al., *EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation.* J. Immunol., 1998. **161**: p. 3096-3102.
12. Forster, R., et al., *CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs.* Cell, 1999. **99**(1): p. 23-33.
13. Baggiolini, M. and P. Loetscher, *Chemokines in inflammation and immunity.* Immunol. Today, 2000. **21**: p. 418-20.
14. von Andrian, U.H. and C.R. Mackay, *T-cell function and migration. Two sides*

of the same coin. N Engl J Med, 2000. **343**(14): p. 1020-34.
15. Lanzavecchia, A. and F. Sallusto, *Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells.* Science, 2000. **290**(5489): p. 92-7.
16. Spergel, D.J., et al., *Using reporter genes to label selected neuronal populations in transgenic mice for gene promoter, anatomical, and physiological studies.* Prog Neurobiol, 2001. **63**(6): p. 673-86.
17. Mancardi, S., et al., *Lymphatic endothelial tumors induced by intraperitoneal injection of incomplete Freund's adjuvant.* Exp Cell Res, 1999. **246**(2): p. 368-75.