人外周血树突状细胞体外诱导扩增的研究

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摘要 目的：探讨可用于临床治疗功能成熟的 DC 体外扩增的优化培养方案。方法：胎牛血清培养基联合细胞因子 rhGM-CSF(100 ng/mL)和 rhIL-4(50 ng/mL)扩增人外周血分离的单个核细胞，细胞培养分别按 5× 10^6/mL、6× 10^6/mL和 7× 10^6/mL的密度，加入 6 孔培养板。第 6d 加入 rhTNF-a(100 ng/mL)联合培养，分别于第 6 d，第 9 d 和 12 d 收获细胞。从形态学、细胞表面标志方面进行鉴定。结果：显微镜观察，经过 9 d 诱导后，培养细胞具有典型树突细胞外形。流式细胞仪分析，6× 10^6/mL密度的细胞培养组培养到第 9 天最宜。结论：细胞具有典型的 DC 的形态特征，细胞表型及功能实验证实其 DC 的特性，说明建立的血清培养基联合细胞因子 rhGM-CSF、rhIL-4 和 rhTNF-a 体外诱导 DC 的方法是切实可行的。

关键词 树突状细胞 细胞培养 细胞因子

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Experimental Study on Induction and Expansion of Dendritic Cells from Human Peripheral Blood In Vitro

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ABSTRACT Objective: To investigate a culture system for DC expansion. Methods: Using serum medium with rhGM-CSF (100ng/mL) and rhIL-4 (50ng/mL) for 6 days, rhTNF-a (100ng/mL) was added on the 6th day of culture. Human peripheral blood were seeding at the concentration of 5× 10^6/mL, 6× 10^6/mL and 7× 10^6/mL in 6-well flat-bottomed plates. Suspension cells were harvested at days 6, 9 and 12 and analysed on morphology, phenotype observed. Result: Cells on day 9 of culture displayed DC-like morphology. Phenotype analysis on DCs acquired showed that the cells of 6× 10^6/mL cultured for 9 days contained a higher percentage of DC cells. Conclusion: The results suggested the culture method was feasible.

Key words: Dendritic cell; Cell Culture; Cytokine

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前言

树突状细胞 (Dendritic cells，DCs) 是 1973 年 Steinman 和 Cohn 在小鼠脾脏贴壁细胞中发现的一种新型细胞，因其表面具有星状多形性或树枝状突起而得名[1]。DC 因其表面具有大量的 MHC 分子和共刺激分子而具有递呈抗原的能力，激发机体的免疫应答，也因此而成为肿瘤免疫治疗的手段之一。然而 DC 数量极少，约占外周血单个核细胞的 1 %，且广泛分布于全身淋巴组织和非淋巴组织中，直接从组织中分离、提取十分困难。DC 体外培养及扩增是 DC 应用于临床的前提条件。本研究旨在探索 DC 体外扩增的优化培养方案。

1 材料和方法

1.1 实验材料

细胞因子 rhGM-CSF 购自美国 R&D Systems 公司，细胞因子 rhIL-4 购自美国 R&D Systems 公司，细胞因子 rhTNF-a 购自美国 R&D Systems 公司，胎牛血清培养基购自中国四季青公司。

1.2 方法

1.2.1 人外周血树突状细胞的培养 采用密度梯度离心法分离外周血单个核细胞。将洗涤后的细胞重悬于胎牛血清培养液中，并加入细胞因子 rhGM-CSF 100 ng/mL，rhIL-4 50 ng/mL调细胞密度为 5× 10^6/mL、6× 10^6/mL和 7× 10^6/mL加入 6 孔板每孔 2mL，置于 37℃，5 % CO_2 培养箱。培养第 6 d，换液时同时加入细胞因子 rhTNF-a 100 ng/mL，继续培养。

1.2.2 人外周血树突状细胞的鉴定 分别于第 6 d、9 d、12 d 收集悬浮细胞，采用流式细胞仪对细胞表面分子 CD1a 鉴定。

1.2.3 统计分析方法 以 Epidata3.0 建立数据库，采用 SPSS 13.0 进行分析，所有数据采用均数 + 标准差表示。

2 结果

2.1 树突状细胞形态观察

从外周血获得单个核细胞，贴壁 2h 后，去悬浮细胞，获得的贴壁细胞即单核细胞。加入细胞因子 rhGM-CSF 100 ng/mL，rhIL-4 50ng/mL。培养到第 6 d 加入细胞因子 rhTNF-a 100 ng/mL，培养到第 9 d 可见大部分细胞悬浮不贴壁，胞质变大，

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形态不规则。培养板上仅有少量贴壁，形态拉长的巨噬细胞（图1）。

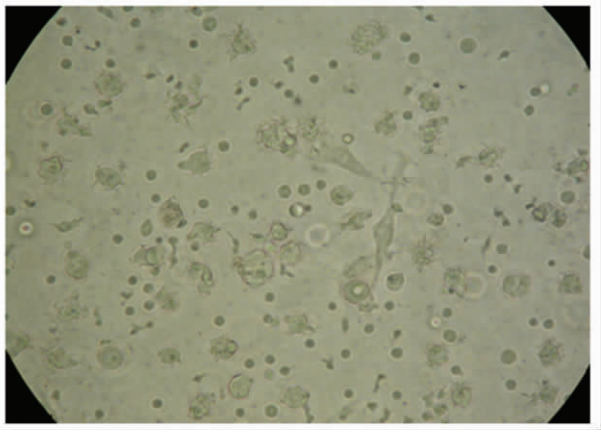

图1 第9天细胞形态

Fig.1 The morphology of cell at 9 day

2.2 细胞培养密度和培养时间的细胞表型比较

细胞培养密度 5× 10^6/mL，6× 10^6/mL和 7× 10^6/mL分别于第 6 d，9 d，12 d 通过流式细胞仪检测 DC 特异的细胞表面标志分子 CD1a，发现细胞密度 6× 10^6/mL和培养到 9 d 时，培养 DC 的效果最好（表 1）。

3 讨论

DC 起源于骨髓多功能造血干细胞[2]，是迄今发现的功能最强的抗原呈递细胞(antigen-presenting cell,APC)，其激发 T 细胞增殖能力及抗原呈递能力是巨噬细胞和 B 细胞的 100～1000 倍[3]。而且 DC 是体内唯一能够活化 Naive T 细胞的 APC，并能特异性的激活细胞毒性 T 细胞，在抗感染、抗肿瘤和治疗自身免疫性疾病等过程中发挥了重要作用[4-8]。然而，尽管 DC 在体内广泛分布于除脑以外的各个脏器[9-11]，但数量少(仅占外周血单个核细胞的 1%左右)且分散，不能满足体内或体外研究及临床应用的需要。因而在体外利用前体细胞对未成熟 DC 进行扩增有重大意义[12]。

DC 表面表达丰富的免疫分子，不同种属的 DC 其细胞表面特异性标志物并不相同[13]。CDla 是目前国内外鉴定人 DC 常用的主要分子。CDla 主要表达于人胸腺细胞、DC 细胞表面，是鉴定 DC 的最好标记[14-18]。本研究中在培养时我们发现初始细胞的浓度对于集落的形成也很重要。我们对不同培养细胞密度比较发现培养细胞密度 6× 10^6/mL，使 DC 前体细胞能互相接触有利于集落的形成但又不至于太拥挤互相抑制生长。我们对不同的培养时间比较发现培养到第 9 天，培养细胞其表面共刺激分子 CD1a 的表达率最高。

表1 细胞表面标志 CD1a 分析

Table 1 Analysis of Phenotype CD1a of cells

Culture density	Culture time		
	At 6 day CD1a(%)	At 9 day CD1a(%)	At 12 day CD1a(%)
5× 10^6/mL	10.83 + 1.50	30.69 + 4.42	16.25 + 2.81
6× 10^6/mL	37.15 + 2.72	65.00+ 5.27	27.05 + 0.52
7× 10^6/mL	34.43 + 0.53	46.95 + 1.36	19.85 + 1.74

综合培养密度和培养时间考虑，以细胞密度 6× 10^6/mL和培养时间 9 天为宜。本研究的 DC 培养方案为进一步研究及临床应用打下了基础。

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要密切监测肝、肾功能，根据病情及时调整剂量，把肝损害的危险程度降至最低；尽量不要与其他药物合用，避免药物相互作用。由于儿童用药不能进行临床试验，所以对儿童用药一定需要特别谨慎。

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