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人表皮细胞培养中Langerhans细胞变化的研究

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提 要 研究表明表皮Langerhans细胞(Lcs)是一种重要的抗原递呈细胞，其参与同种皮肤排斥反应[1][2]。如消除移植物内Lcs，可延长异体皮肤存活[3-6]。本研究系利用ATPase染色，抗人HLA-Dr抗原单克隆抗体间接免疫荧光及免疫酶标（ABC）染色，观察了人表皮细胞（Ecs）培养中Lcs的密度及形态变化。并通过混合表皮细胞淋巴细胞培养反应（MEcLR），检查了培养前后Ecs的免疫刺激能力。发现在Ecs培养过程中，存于Ecs内的Lcs培养3天即显著减少同时明显变形；7天后Lcs减少达90%以上；培养14天或更长时间Ecs内Lcs基本消失。在培养新生的Ecs内自始未发现Lcs。培养7天后的Ecs及新生Ecs在MEcLR中失去刺激异体淋巴细胞转化的能力。这些结果表明通过Ec体外培养可以消除Ecs内携带Ⅰ级抗原的Lcs，提示本培养系统培养的同种人Ecs如用于异体移植，其存活的时间可能延长。

关键词 人表皮细胞培养；Langerhans细胞；混合表皮细胞淋巴细胞反应

人表皮Lcs源于骨髓，是Ecs内唯一具有Fc-IgG受体，C_3受体及HLA-Dr抗原的细胞亚群[7-8]。Lcs参与皮肤细胞介导的免疫反应，与同种皮肤排斥反应有关[2]，如能消除移植物内Lcs，可延长异体皮肤存活[3-6]。近年来虽然有不少学者报告培养的同种人Ecs用于异体移植，获得成功[6][9]，然而Ecs体外培养对Lcs有何影响，通过体外培养是否能消除Ec内Lcs，却是未曾探索的问题。为此我们进行这方面的研究，试图为临床上大量开展同种人Ecs培养并用于烧伤创面覆盖提供线索。

材料和方法

一、皮肤标本及Ecs培养 按我室曾报告的方法进行人Ec培养。从外科手术切除物如乳腺、截肢、包皮等得外观正常皮肤，去尽皮下组织后剪成细小碎块，均匀接种于平皿。加Eagl-MEM培养液，置CO_2孵箱培养。

二、接种表皮组织块及培养新生Ec内Lcs的鉴定 于培养0、3、7、14、21天时，从接种表皮组织块分离全层Ec及周围新生Ec单层进行下列各项检查：

1．三磷酸腺苷酶（ATPase）染色：根据Juhlin等的方法[10]并作部分修改。待测皮片直接放入ATP-铅溶液内，37℃孵育60分钟。经2%硫化铵浸染后，贴片（真皮侧面向上），光镜下观察计数。

2．抗人HLA-Dr单抗间接荧光染色：皮片经丙酮固定后，PBS（0.01M，pH7.2）漂洗2′×3次。加鼠抗人HLA-Dr单体（由军事医学科学院基础医学研究所提供），4℃孵育过夜，1:40FITC-羊抗鼠IgG血清，37℃孵育60分钟。贴片，荧光镜下观察计数。

3．亲和素-生物酶标复合物（ABC）染色：为了与荧光染色对照检查，故采用了ABC染色。所用生物素化兔抗鼠IgG（RAM-G-6）、生物素化辣根过氧化物酶（b-HRP）及亲和素均购自上海生物制品所。方法同于荧光染色。

4．ATPase、HLA-Dr阳性Lcs的计数方法：在显微镜目镜内装置一方格式微型计数器，先测其面积，据任意10个视野内阳性Lcs数，计数所测皮片Lcs的密度，以Lcs/mm²表示。

三、混合表皮细胞淋巴细胞培养反应（MEc LR） 按Pollack等[11]的方法并作部分修改。从不同培养时间之表皮组织块及培养新生Ec单层分离Ec；从健康自愿者静脉血分得单个核细胞。每只样管加5×10^5个Ec及5×10^5个单个核细胞混合培养。培养7天后加^{3}H-TdR 3微居（μCi），继续培养18小时取出。抽滤，洗尽未掺入的^{3}H-TdR。测定反应细胞掺入^{3}H的量，以cpm表示。

实验结果

一、Ec的生长情况及形态观察

培养3～5天接种表皮组织块边缘长出新生Ec集落，以后逐渐向四周扩展。2～3周新生Ec融合成片，新生Ec胞体大，胞浆透明

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（图1）。

二、培养过程中Lc密度及形态变化

1．接种表皮组织块内Lc密度及形态：

1）ATPase染色：在未经培养的表皮组织块内可见密集分布的ATPase阳性Lc，阳性细胞呈棕黑色，有典型的树枝状胞突（图2）。培养过程中Lc逐步退变，数量减少。培养3天，表皮内Lc胞突即变短或消失；7天后Lc大部分消失，残存Lc呈破溃状圆形细胞残碎屑状ATPase阳性物质（图3）。培养14天或更长时间仅个别标本内偶见Lc的残迹。Lc的密度改变见表1。

表1 不同培养时间表皮组织内Lcs的密度（N＝15）

	培养时间（天）				
	0	3	7	14	21
ATPase染色	746.4±78.3#	173.7±38.3*	69.7±25.9*	23.3±13.2*	10.4±7.5*
HLA-Dr荧光染色	858.3±77.3	198.6±53.3*	18.3±10.8*	6.7±2.3*	0
ABC 染色△	799.4±49.3	161.4±50.2*	12.3±7.7*	0	0

注：#：M±SD　　*：方差分析差别非常显著（P＜0.001）

△：N＝10

2）HLA-Dr单抗间接免疫荧光染色：

未经培养的表皮组织内见密密集集星芒状HLA-Dr阳性Lc（图4）。培养3天后，HLA-Dr阳性细胞明显减少，胞突消失（图5）。7天后阳性细胞进一步减少，残存Lc胞体增大，边界模糊呈聚合的团块状荧光；14天后HLA-Dr阳性Lc消失。

3）HLA-Dr单抗ABC染色：染色阳性细胞呈棕黄色，有特征性的树枝状胞突（图6）。随培养时间延长，阳性Lc逐步减少，残存Lc退变以至消失。

2．培养新生Ec内Lc的鉴定：培养过程中，在接种表皮组织块周围新生的Ec内，未发现ATPase、HLA-Dr阳性Lc（图7）。

三、培养前后Ec在MEcLR中刺激异体淋巴细胞转化的能力

1．不同培养的时间表皮组织块之Ecs在MEcLR中刺激异体淋巴细胞转化的能力：结果见表2。从表2可见，未经培养的新鲜Ec，能强烈刺激异体淋巴细胞转化。培养时间延长，Ec的刺激能力减弱，以至丧失其刺激能力。将培养7天的Ec在MEcLR中所得结果与新鲜Ec及培养3天的Ec比较皆相差显著（P＜0.001），与单纯新鲜Ec或淋巴细胞培养的对照管比较无显著差别（P＞0.05），说明培养7天后的Ec在MEcLR中已不具有刺激异体淋巴细胞转化的能力。

表2 经不同时间培养之Ec在MEcLR中刺激异体淋巴细胞转化的能力

		培养时间（天）		
		0	3	7
MEcLR(cpm),△	$\bar{X}$	17931.4	4030.1	550.6#
	SE	1884.9	561.8	75.1
对照管:*△	$\bar{X}$	568.9	445.6	417.6
	SE	79.5	68.1	36.9

注：# t检验与未培养Ec及培养3天的Ec比较P＜0.001；与对照管比较P＞0.05。

* 单纯Ec或淋巴细胞培养；△ N＝10

2．培养的新生Ec在MEcLR中刺激异体淋巴细胞转化的能力：结果见表3。从表3可见培养的新生Ec在MEcLR中不具有刺激异体淋巴细胞转化的能力，其结果与单纯Ec或淋巴细胞培养的对照管比较无显著性差别（P＞0.05）。

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表3 未经培养的Ec与培养新生的Ec在MEcLR中刺激异体淋巴细胞转化能力

皮标本	未经培养的Ec		培养新生的Ec	
	淋巴细胞掺入^{3}H-TdR量(cpm)	对照管△	淋巴细胞掺入^{3}H-TdR量(cpm)	对照管△
$\bar{X}$	16239.6	444.5	673.8*	497.8
SE	653.6	55.9	67.3	41.2
N	8	8	8	6

注：△ 单纯Ec或淋巴细胞培养；* t检验与未经培养的Ec比较$P<0.001$；与对照管比较$P>0.05$

讨 论

表皮Lc源于骨髓并由骨髓来源的Lc的前身细胞不断补充更新[12]，其自身增殖是极其罕见的[13]，人表皮Lc携带的HLA-Dr抗原（相当于小鼠H-2 Ia抗原）是重要的移植抗原，在激发同种皮肤发生免疫排斥方面起关键作用[2]，不少作者报导用紫外线照射，类固醇激素处理的皮肤可延长异体皮肤存活，认为与表皮内Lc被清除有关[3-6]。

本研究利用表皮组织块接种法进行Ec培养，观察了人Ec培养中Lc的形态及功能变化。实验发现，无论是从Lc的ATPase活性或是从其膜表面HLA-Dr抗原检查结果，随培养时间延长，接种表皮组织块内Lc皆呈一致性减少，以至最后Ec内Lc消失。同时观察到，培养中Lc不但数量逐步减少，而且残存Lc明显变形，其胞突变短、消失；胞体变形呈崩溃状，直至最后Lc的形态模糊、消失，说明培养中Lc减少或消失不是Lc某一膜标志的变化，而是Lc本身消失的结果。

Morhenn[14]及Hefton[15]等将经胰蛋白酶分散的人Ec进行培养，发现培养7～14天后的Ec内不再有携带HLA-Dr抗原的Lc，与本研究观察的结果基本一致。

本研究还发现，培养中存于接种表皮组织块内的Lc既无增殖，也不迁移到周围新生的Ec中去，在培养新生的Ec内自始未发现有ATPase、HLA-Dr阳性Lc。

MEcLR可反映两个同种个体之间的组织不相容性程度，并已作为临床器官移植的一项组织配型方法[16]。我们从不同培养时间之表皮组织块分得Ec进行MEcLR，发现未经培养的新生Ec能强烈刺激异体淋巴细胞转化。培养7天以后的Ec在MEcLR中失去刺激功能。同时发现培养新生Ec在MEcLR中不具有刺激异体淋巴细胞转化的能力。说明培养7天后的Ec及新生Ec二者组织不相容性程度减弱。

Stingl等[17]用梯度离心法将Ec分离成富含Lc的Ec及去除Lc的Ec，分别进行MEcLR，发现富含Lc的Ec能强烈刺激异体淋巴细胞转化，而去除Lc的Ec失去其刺激能力。本研究培养7天后的Ec及培养新生Ec在MEcLR中失去刺激异体淋巴细胞转化的功能，提示培养后及培养新生的Ec内Lc被清除。这点也与前述培养中Lc密度变化的观察结果相印证。

皮肤移植内Lc密度与其本身存活时间有密切关系。Streilein等[18]将缺乏Lc的小鼠角膜异位移植到仅H-2.I区不相一致的同种动物体壁，发现其存活45天尚未排斥；并进一步证实含Lc密度较低的小鼠尾部皮肤同种移植后能明显延长其存活时间[2]。Alsbjörn等[4-5]用紫外线照射人皮肤，发现可使局部Lc减少90%以上，将此含Lc密度较低的皮肤移植给烧伤病人，其存活100天尚存在。Hefton等证实培养7～14天后的人Ec内没有Lc，将此Ec移植到烧伤切痂创面存活9个月之久尚未发生排斥[6]。本研究观察到在Ec培养过程中存在于接种表皮组织块内的Lc逐步减少以至消失，同时培养新生的Ec内也没有携带HLA-Dr抗原的Lc，说明本培养系统培养的人Ec如用于异体移植，延长其存活时间是有可能的。

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• Abstracts •

Experimental Study on Target Cells of Pseudomonas aeroginosa Exotoxin A

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Pseudomonas aeroginosa exotoxin A(PEA) was purified from the strain PA-103 and labelled with I^{125}. Then autoradiographic tracing, histopathological observations with light and electron microscopes, and antiserum protestion test were carried out on inbred mice BALB/C. The results indicate that the liver is the target organ of PEA, and both the hepatocytes and kupffer cells the target cells, on which the severe damage may be responsible for the acute death of the toxicated animals.

Key words: Pseudomonas aeroginosa exotoxin A; Target cell; Hepatocyte; Kupffer cell (p.1)

Study of the Changes of Langerhans in Cultured Human Epidermal Cells

Zhang Shijun, et al(Burn Center, Third Miliitary Medical College)

It has been shown that epidermal Langerhans cells (LCs) were antigen presenting cells in cutaneous cell-mediated immuneresponse, and they were implicated in skin allograft rejection[1][2]. If LCs can be depleted from skin grafts, the survival period of the allografts may be prolonged[3~6]. Therefore, we observed the changes of LCs in cultured human epidermal cells (ECs) by ATPase staining, HLA-Dr monoclonal antibody indirect immunofluoresence and enzyme labelling avidin-biotin complex (ABC) staining. And the capacity of ECs to stimulate allogeneic lymphocyte proligeration before and after cultures was tested by mixed epidermal cell-lymphocyte culture response (MEcLR). We found that LCs in original skin explants were significantly decreased and deformed three days after culture. LCs were reduced more than 90 percent after 7 days of culture. LCs in original skin explants were approximately eliminated 14 days or more of culture. LCs were never seen within epidermal outgrowth in culture. The Ecs gained from the original skin explants which were cultured after 7 days and from epidermal outgrowth lost their ability to stimulate allogeneic lymphocyte transformation in MEcLR. These results indicate that LCs bearing HLA-Dr

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antigen in human are able to be depleted from epidermal grafts by epidermal cell culture, and suggest that the ECs cultured in our culture system can prolong their survival period as they are used as allografts.

Key words: Culture of human Human Epidermal cells; Langerans cells; Mixed epidermal cell-lymphocyte cnlture response (p.5)

Ultrastructural Pathology of Experimental Encephalitis B and Morphogenesis and Release of Encephalitis B Virus in Mice

Chen Liming Tutors: Yan Liansui and Liang Yanjie (Department of Pathology, Third Military Medical College)

An animal model of encephalitis B was successfully established with Jin Wei Yan I strain virus in mice, which made it possible to study the ultrastructural pathology as well as the morphogenesis and release of encephalitis B exerimentally.

The ultrapathological changes occurring to the brain tissues especially in the neuron cells were described systematically. A preliminary hypothesis about the morphogenesis and release of encephalitis B virus (Jin Wei Yan I strain) was proposed on the basis of these changes. Replication of the virus was also found to occur in the oligodendroglia but not in the microglia, which maintained its phagocytic function. This fact evidently shows that the micrioglia is not controlled by the mRNA of the virus.

According to the literature available to the authors, it seems that the ultrastructural pathological changes of encephalitis B with typical histological lesions in animals had not been reported previously.

Key Words: Encephalitis B virus; Ultrastructural pathology; Encephalitis B (p.9)

Effects of Activated Complement on Lung Fluid Exchange in Conscious Goats: Dependency on Superoxide Anion

Xie Zhonglin, et al (Department of Pathophysiology and Institute of Hypoxia, Third Military Medical College)

The effects of zymosan-activated plasma (ZAP) on lung fluid exchange and superoxide anion production by granulocytes were studied in 8 conscious goats with chronic lung lymph fistula. It was found that the circulating granulocytes were activated and the superoxide anion production was markedly enhanced af-

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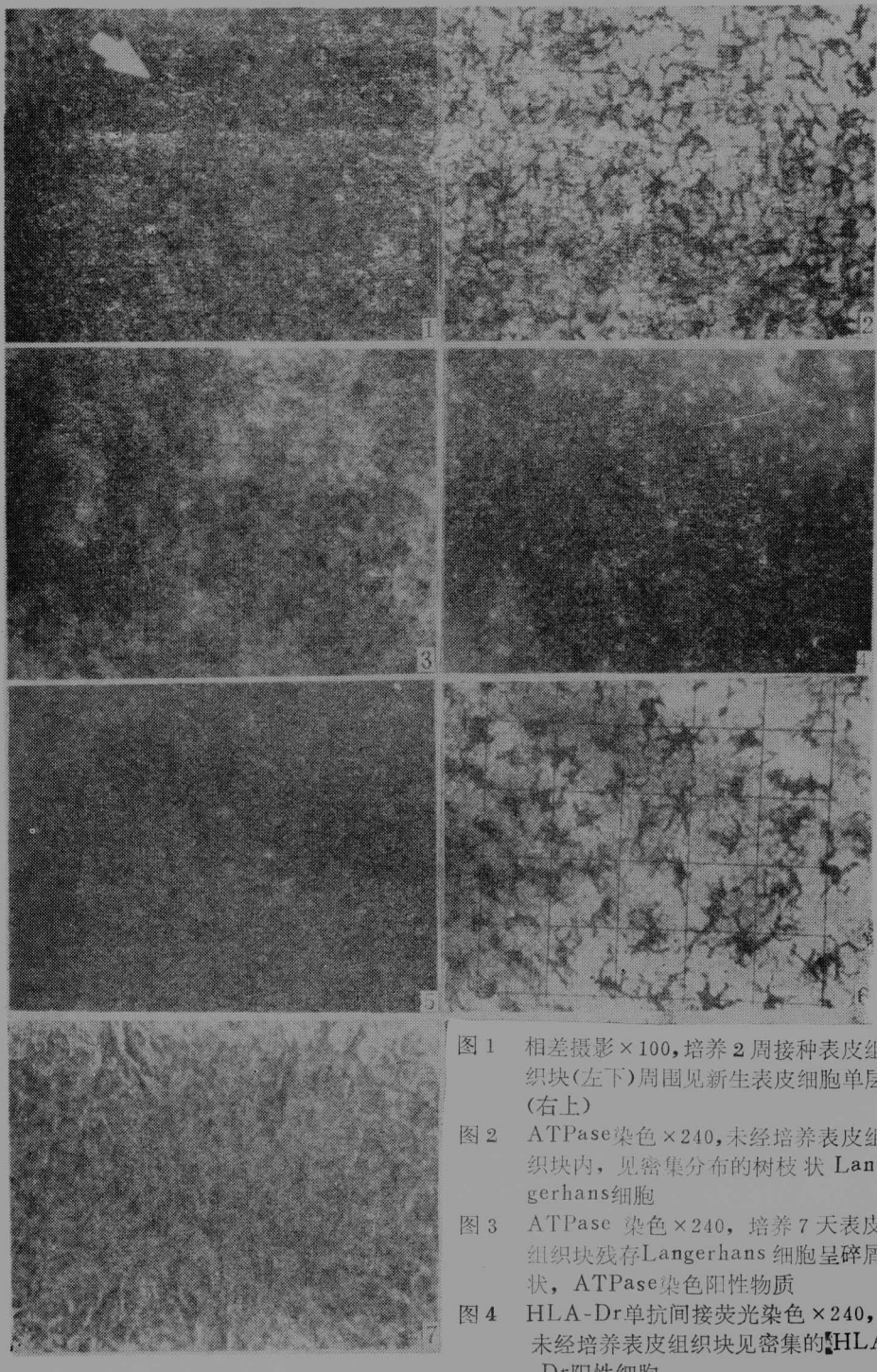


图1 相差摄影×100，培养2周接种表皮组织块(左下)周围见新生表皮细胞单层(右上)

图2 ATPase染色×240，未经培养表皮组织块内，见密集分布的树枝状Langerhans细胞

图3 ATPase染色×240，培养7天表皮组织块残存Langerhans细胞呈碎屑状，ATPase染色阳性物质

图4 HLA-Dr单抗间接荧光染色×240，未经培养表皮组织块见密集的HLA-Dr阳性细胞

图5 HLA-Dr单抗间接荧光染色×240，培养3天表皮组织块内HLA-Dr阳性细胞减少；残存Lc树枝状胞突消失

图6 HLA-Dr单抗ABC染色×240，未经培养表皮组织块内见典型的树枝状Lc

图7 HLA-Dr单抗ABC染色×240，培养新生的Ec单层内未发现阳性细胞

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