DOI:10.13471/j.cnki.j.sun.yat-sen.univ(med.sci).2000.0008

肺癌患者肺泡巨噬细胞表现 HLA-DR 的表达

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摘要:【目的】了解肺癌患者肺泡巨噬细胞表面人白细胞抗原-DR(HLA-DR)的表达。【方法】经支气管肺泡灌洗获肺泡巨噬细胞,贴壁分离及培养,免疫组化方法检测 HLA-DR 表达的阳性细胞百分率。【结果】① 肺癌及良性肺病患者,其肺泡巨噬细胞未经刺激,均已有部分表达 HLA-DR,但前者明显高于后者;② 干扰素-α(IFN-α)或脂多糖(LPS)刺激后,HLA-DR 的表达均增加,且两者联合刺激优于单独刺激;③ 无论刺激与否,肺癌组荷瘤侧肺与非荷瘤侧肺比较,肺泡巨噬细胞表达 HLA-DR 的阳性细胞率明显增高。【结论】肺癌患者,尤其是肿瘤局部,肺泡巨噬细胞可能通过表达 HLA-DR 提高抗原递呈功能。IFN-α、LPS 均能明显增强肺癌患者肺泡巨噬细胞的抗原提呈功能,且两者有协同作用。

关键词:肺肿瘤;巨噬细胞,肺泡;HLA-DR 抗原

中图分类号:R 734.2 文献标识码:A 文章编号:1000-257X(2000)01-0024-03

Expression of HLA-DR on Alveolar Macrophages from Patients with Lung Cancer

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Abstract:【Objective】To observe the expression of human leukocyte antigen DR (HLA-DR) on alveolar macrophages.【Methods】The alveolar macrophages obtained by bronchoalveolar lavage, were separated and incubated. The expression of HLA-DR was detected and the positive rates of alveolar macrophages were compared.【Results】① Alveolar macrophages from all cases express HLA-DR without stimulation. The positive rate of alveolar macrophages was higher from patients with lung cancer than that from cases with nonmalignant pulmonary diseases. ② Expression of HLA-DR in case after stimulation with IFN-α or LPS were higher than that wihtout stimulation, and stimulation with IFN-α and LPS in combination was the highest. ③Whether stimulated or not, the positive rate of alveolar macrophages from the tumor-bearing side of lung was higher than that from the nontumor-bearing side of lung.【Conclusion】The funtion that alveolar macrophages submit antigen was enganced, because the alveolar macrophages aroud the tumor express HLA-DR. The function that alveolar macrophages submit antigen was enhanced after stimulated with IFN-α or/and LPS, and the effection of IFN-α and LPS was cooperative.

Key words: lung neoplasms; macrophage, alveolar; HLA-DR antigen

活化的巨噬细胞具有抗肿瘤功能。判断巨噬细胞激活的两个特征指标是表达抗原提呈功能和巨噬细胞介导的肿瘤细胞毒功能。抗原提呈功能以免疫相关抗原(Ia)为效应分子,在人类既为人白细胞抗原 DR(HLA-DR)。我们通过检测肺癌患者肺泡巨细胞表面 HLA-DR 的表达,探讨肺癌患者肺

收稿日期:1999-06-21

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泡巨噬细胞的抗原提呈功能及其影响因素。

1　材料与方法

1.1　试　剂

抗 HLA-DR 单克隆抗体(MA-H HLA-DR), 丹麦 DAKO 公司产品。链霉菌抗生物素蛋白-过氧化物酶染色(SP 法[1])试剂盒为福州迈新生物技术公司产品。干扰素-α(IFN-α), 美国 SCHRING 公司产品; 脂多糖(LPS), 湖南医科大学免疫室提供。

1.2　肺泡巨噬细胞的分离及培养

对 32 例肺癌患者(病理证实)的右中叶(25 例)或左舌叶(7 例)行支气管肺泡灌洗, 其中荷瘤侧肺 14 例, 非荷瘤侧肺 18 例; 28 例良性肺病患者均行右中叶的支气管肺泡灌洗。收集支气管肺泡灌洗液, 按贴壁培养的方法进行分离, 获纯度在 95%以上的肺泡巨噬细胞。分 4 瓶(2×10^6/瓶), 分别加入 RPMI-1640、IFN-α(0.25×10^6 U/L)、LPS(250 mg/L)、IFN-α(0.25×10^6 U/L)加 LPS(250 mg/L)各 2 mL, (37 ℃、5% CO_2)培养 24 h, 收集细胞, 取出含 1×10^6 个细胞的悬液, 置−70 ℃保存。

1.3　肺泡巨噬细胞表面 **HLA-DR** 的检测

用免疫组化 SP 法, 按链霉菌抗生物素蛋白-过氧化物酶染色试剂盒提拱的操作程序进行, 高倍镜下计数 200 个细胞, 计表达 HLA-DR 的阳性肺泡巨噬细胞百分率。

1.4　统计学方法

采用两因素方差分析。

2　结　果

表 1　肺癌与良性肺病患者支气管肺泡灌洗液的各项指标

Table 1　Bronchoalveolar lavage fluid from patients of lung cancer or nonmalignant pulmonary disease　($\bar{x}\pm s$)

Group	Cases (n)	Rate of reclamation(%)	Total number of cells(10^7)	Percent of alveolar macrophage(%)	Livability of alveolar macrophage(%)
Lung cancer Nonmalignant	32	56.3±11.2	3.4±2.0	93.8±2.9	89.5±3.1
Pulmonary Disease	28	60.8±8.7	3.7±0.9	94.2±2.8	88.5±4.8
P		0.0　>0.10	0.0　>0.10	0.0　>0.40	0.0　>0.40

肺癌与良性肺病组比较, 支气管肺泡灌洗液的回收率、有核细胞总数、肺泡巨噬细胞百分率、肺泡巨噬细胞活率均无显著性差异。

表 2　肺癌与良性肺病患者肺泡巨噬细胞表达 HLA-DR 的阳性细胞率

Table 2　The positive rate of alveolar macrophages express HLA-DR from patient of lung cancer or nonmalignant pulmonary disease　(%)

Group	Cases(n)	Without stimulation	IFN-α	LPS	IFN-α + LPS
Lung cancer	32	46.2±5.3	60.3±5.8	61.3±5.8	72.6±6.4
Nonmalignant Pulmonary Disease	28	18.3±4.0	27.7±5.4	28.9±4.6	38.5±5.8
P		<0.001	<0.001	<0.001	<0.001

肺癌组与良性肺病组比较, 在无或有 IFN-α 和 LPS 刺激下, 肺泡巨噬细胞表达 HLA-DR 阳性细胞率均有显著性差异; 无论肺癌组或良性肺病组, IFN-α 或/和 LPS 刺激较未刺激的肺泡巨噬细胞, 其阳性细胞率均有显著性差异(均为 $P<0.01$); 两者联合刺激与单独刺激比较, 其阳性细胞率有显著性差异(均为 $P<0.01$), 但两者单独刺激比较, 差异无显著性($P>0.05$)。

表 3 肺癌患者荷瘤侧肺与非荷瘤侧肺肺泡巨噬细胞表达 HLA-DR 的阳性细胞率

Table 3 The positive rate of alveolar macrophages that express HLA-DR from tumor-bearing side or nontumor-bearing side of lung cancer (%)

	Cases(n)	Without stimulation	IFN-α	LPS	IFN-α+LPS
Tumor-bearing side of lung	14	49.00±5.12	63.00±5.98	64.43±4.55	76.14±4.07
Nontumor-bearing side of lung	18	44.25±4.65	58.14±7.18	58.86±5.64	69.92±6.58
P		<0.02	<0.05	<0.01	<0.005

3 讨 论

肿瘤组织临近的肺泡巨噬细胞活性目前尚有争论。有资料表明,肿瘤细胞培养上清液能激活单核-巨噬细胞[2];Hengst 等报道,肺癌患者肺泡巨噬细胞能显示自发性细胞毒活性,推测肺泡巨噬细胞可被慢性局部病变(如肺癌)所激活,而非先天具有自发性细胞毒活性[3]。我们的实验结果显示,肺癌患者肺泡巨噬细胞在体外培养 24 h,即使不刺激,亦有部分肺泡巨噬细胞表达 HLA-DR,其阳性细胞率明显高于良性肺病组,且肺癌组荷瘤侧肺明显高于非荷瘤侧肺。单核-巨噬细胞提呈抗原的功能与细胞表面的 HLA-DR 抗原量有直接关系[4],说明肺癌患者,尤其是肿瘤局部,肺泡巨噬细胞的抗原提呈功能是明显增强的。LPS 及 IFN-α 均为巨噬细胞激活物。我们的实验结果显示,LPS 及 IFN-α 均能增加肺癌组及良性肺病组 HLA-DR 阳性的肺泡巨噬细胞百分率,且肺癌组明显高于良性肺病组;两者联合刺激较单独刺激比较,肺泡巨噬细胞表达 HLA-DR 阳性细胞率明显增高。说明 LPS、IFN-α 均能明显增强肺癌患者肺泡巨噬细胞的抗原提呈功能,且两者有协同作用。

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(编辑 黄小延)

(上接第 23 页)

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