P.O.Box 2345 Beijing 100023, China
Fax: +86-10-85381893
Email: wcjd@wjgnet.com www.wjgnet.com

World Chin J Digestol 2003 September;11(9):1375-1377
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R

IL-4增强IL-2活化的A-NK细胞对人直肠癌CC95的抗肿瘤作用

王志华, 申宝忠, 史 历

王志华, 史历, 哈尔滨医科大学肿瘤研究所 黑龙江省哈尔滨市 150040
申宝忠, 哈尔滨医科大学第三临床医学院 黑龙江省哈尔滨市 150040
王志华, 男, 1951-03-05生, 黑龙江省哈尔滨市人, 汉族, 1978年毕业于哈尔滨医科大学, 研究员, 主要从事肿瘤免疫学和肿瘤分子生物学研究.
国家自然科学基金课题, No.39270765
黑龙江省重点攻关课题, No.G00C190401
项目负责人: 王志华, 150040, 黑龙江省哈尔滨市南岗区哈平路150号, 哈尔滨医科大学肿瘤研究所. hljwzh@163.com
电话: 0451-2620314 传真: 0451-6665003
收稿日期: 2002-12-10 接受日期: 2002-12-26

IL-4 enhances antitumor effect of IL-2 induced A-NK Cells on human colon carcinoma

Zhi-Hua Wang, Bao-Zhong Shen, Li Shi

Zhi-Hua Wang, Li Shi, Cancer Research Institute, Harbin Medical University, Harbin 150040, Heilongjiang Province, China
Bao-Zhong Shen, The Third College of Clinical Mediccine, Harbin Medical University, Harbin 150040, Heilongjiang Province, China
Supported by the National Natural Science Foundation of China, No. 39270765 and the Key Programs of Foundation of Heilongjiang Province, No.G00C190401
Correspondence to: Dr Zhi-Hua Wang, Cancer Research Institute, Harbin Medical University, 150 Haping Road, Harbin 150040, Heilongjiang Province, China. hljwzh@163.com
Received: 2002-12-10 Accepted: 2002-12-26

Abstract

AIM: Using IL-4 combined with IL-2 to induce A-NK cells and to evaluate the cytotoxicity of A-NK cells and its inhibiting effect on colon tumor growth.

METHODS: The A-NK cells were activated with recombinant lymphokine IL-2 combined IL-4, the cytotoxicity of the effector cells was determined by LDH-L release assay. Its antitumor effect was investigated through growth inhibiting of human colon carcinoma cells in nude mice.

RESULTS: IL-2 alone or combined with IL-4 could induce the activity of A-NK cells successively which could kill K562, Anip973 and CC95 tumor cells *in vitro* by LDH-L release assay (39.00 ± 9.16 *vs* 77.68 ± 12.80, 43.10 ± 10.05 *vs* 80.02 ± 13.74, 42.14 ± 9.72 *vs* 79.10 ±12.65, P <0.01) and inhibit the growth of human colon carcinoma cells in nude mice (1.04 ± 0.15 *vs* 0.62 ± 0.16, P <0.01).The results suggested that there was expression of IL-4 receptor on the surface of A-NK cells.

CONCLUSION: IL-4 can enhance the antitumor activity of IL-2 induced A-NK cells. The method may have some potential application value in human cancer treatment.

Wang ZH, Shen BZ, Shi L. IL-4 enhances antitumor effect of IL-2 induced A-NK Cells on human colon carcinoma. Shijie Huaren Xiaohua Zazhi 2003;11(9):1375-1377

摘要

目的: 用IL-4联合IL-2共同诱导A-NK细胞, 研究A-NK细胞体外细胞毒性及对直肠肿瘤生长的抑制作用.

方法: 用基因重组的IL-2和IL-4联合活化A-NK细胞, 用LDH-L释放法测定效应细胞的细胞活性, 同时观察A-NK细胞对人直肠癌细胞在裸鼠体内的生长抑制作用.

结果: IL-2单独或IL-2联合IL-4均可以诱导A-NK细胞, 但二者联合效果更好, 通过LDH-L测定活化的A-NK细胞可在体外杀伤K562, Anip973和CC95细胞(39.00±9.16 *vs* 77.68±12.80, 43.10±10.05 *vs* 80.02±13.74, 42.14±9.72 *vs* 79.10±2.65, P <0.01), 抑制人直肠癌肿瘤在裸鼠体内的生长(1.04±0.15 *vs* 0.62±0.16, P <0.01), 提示A-NK细胞膜表面存在IL-4受体的表达.

结论: IL-4能够增强IL-2诱发的A-NK细胞抗肿瘤活性, 此方法在将来肿瘤临床治疗中具有潜在的应用价值.

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http://www.wjgnet.com/1009-3079/11/1375.asp

0 引言

A-NK细胞(activated natural killer cell)具有独特的生物学特性, 与常规LAK或NK细胞相比有更好的抗肿瘤作用[1-6]. 目前, 国际上A-NK细胞诱导和活化多采用IL-2单独进行[7-11]. 我们分别应用IL-2单独或IL-2和IL-4联合诱导A-NK细胞, 观察了该细胞体外细胞毒活性和对直肠裸鼠体内移植癌的抑制作用. 结果提示应用IL-2和IL-4联合诱导的比较IL-2单独诱导的A-NK细胞具有更高的抗肿瘤效果.

1 材料和方法

1.1 材料 RPMI1640培养液来自Gibco公司; LDH-L试剂盒从中国科学院上海生化研究所购买; 基因重组白介素2(rIL-2)是卫生部长春生物制品研究所的产品; rIL-4从日本Nipro公司购买; K562, 人红白血病细胞株由日本北海道大学惠赠; 人肺腺癌细胞株(Anip973)和直肠癌细胞株CC95由本所建立; Balb/c裸鼠, ♀, 5-6周龄, 从中国医学科学院动物部获得, 在无病原微生物的洁净条件下实验; RPMI1640培养液含100 mL/L小牛血

清(BCS)用于肿瘤细胞培养; 培养人A-NK细胞的RPMI 1 640加入100 mL/L的人AB型血清; 两种细胞均在37 ℃, 50 mL/L CO_2 培养箱中培养.

1.2 方法 常规分离脾细胞, 用尼龙毛柱法去除单核细胞后, 用含有100 mL/L人AB血清的RPMI 1640调整细胞浓度为2×10^8/mL. 将细胞悬液分3组移至培养板中, 第1组, 不加细胞因子, 即脾细胞; 第2组, 单独加IL-2 (500 kU/L)诱导; 第3组, 加入IL-2 (500 kU/L)和IL-4 (500 kU/L)联合诱导. 连续培养3 d后用于实验. 效应细胞的细胞毒性采用LDH-L释放测定法. 三种靶细胞(K562, Anip973, CC95, 各10^8/L)悬液分别加入96孔板, 每孔100 μL, 效应细胞依不同的效靶比例加入.

% 特异溶解 =(实验溶解 cpm - 自发溶解 cpm)/ (最大溶解 cpm - 自发溶解 cpm) × 100 %

将人直肠癌细胞(CC95) 0.1 mL注射至Balb/c裸鼠背部皮下, 动物随机分成3组, 每组5只. 第1组注射脾细胞作为对照; 第2组为IL-2诱导的A-NK细胞; 第3组注射IL-2和IL-4联合诱导的A-NK细胞. 第1, 3, 5 d, 第2, 3组的动物分别静脉注射相应的效应细胞0.2 mL (1×10^{10}/L), 每3 d用卡尺测量肿瘤大小并加以记录.

统计学处理 依实验不同分别采用t检验和χ^2检验, $P<0.05$有统计学意义.

2 结果

2.1 细胞毒性测定 IL-2单独或IL-2联合IL-4都能成功地诱导A-NK细胞活性. 由IL-2和IL-4共同诱发的A-NK细胞比IL-2单独诱发的A-NK细胞的杀伤活性又有显著提高, 对Anip973的杀伤率分别为77.38 %和38 %, 对K562和CC95细胞的杀伤作用与之相似(表1).

表1 IL-4和IL-2诱导的A-NK细胞体外细胞毒性

效应细胞	诱导条件	特异性溶解 (%)		
		Anip973	K562	CC95
脾细胞		4.43±0.25	3.25±0.19	3.76±0.23
A-NK细胞	IL-2单独	39.00±9.16	43.10±10.05	42.14±9.72
A-NK细胞	IL-2+IL-4	77.68±12.80[b]	80.02±13.74[b]	79.10±2.65[b]

E/T = 40/1, ($\bar{x}\pm s$, n =3) [b]$P<0.01$, IL-2单独 *vs* IL-2+IL-4联合诱导组.

表2 IL-4和IL-2诱导A-NK细胞对裸鼠人直肠癌CC95移植肿瘤40 d的抑制作用

组份	肿瘤重量(g)	抑制率(%)
对照组	1.82±0.11	-
IL2单独	1.04±0.15	44.3
IL4+IL-2	0.62±0.16	66.7[b]

[b]$P<0.01$, IL-2单独 *vs* IL-2+IL-4联合诱导组.

2.2 A-NK细胞体内抗肿瘤作用 与对照组相比, 用A-NK细胞治疗裸鼠人直肠移植肿瘤CC95取得了明显的抑制生长的作用, 并且2、3组之间差异显著($P<0.01$, 表2). 结果提示用IL-4和IL-2共同诱导能更好地活化A-NK细胞增强其抗肿瘤作用.

3 讨论

在抗肿瘤免疫中细胞免疫扮演一个重要的脚色. 近来很多针对A-NK细胞的研究提示与普通LAK细胞相比A-NK细胞有着更快的扩增速度, 更高的杀伤效果, 由此吸引了大批研究者的广泛关注[12-16]. Brunson *et al*[17]曾报告用荧光标记、IL-2活化的A-NK细胞过继转输后能选择性的聚集到建立的肺或肝转移灶内, 结合到肿瘤细胞和/或微小血管的内皮细胞上. Smits *et al*[18]指出由于大多数肿瘤细胞表面缺乏CD18的表达, A-NK细胞表面和CD18相关的整合素是溶解靶细胞所必须的.

但是如何进一步提高肿瘤生物治疗的作用, 成功的分离, 诱导A-NK细胞使之具有较大的抗肿瘤治疗潜力, 增加其疗效是一个不容忽视的课题. 愈来愈多的研究提示不同结构的细胞因子有相似或叠加的生物学活性, 结合应用能够产生累计协同作用, 增加治疗效果同时减少用量, 即达到增效, 减毒的效果[19-20].

直肠癌是临床上常见的恶性肿瘤, 发病率逐年上升, 很多研究表明该病的发生和多种癌基因和抑癌基因的改变有关[21-33]. 本研究中, 我们应用IL-4和IL-2联合或IL-2单独诱导A-NK细胞在体外取得了抗K562、Anip973和CC95三种肿瘤细胞的作用, 在裸鼠也成功地抑制了肠癌细胞的生长, 尤其是联合应用的效果更佳, 此结果支持了上述观点, 即联合诱导的效应细胞更加有效.

关于IL-4是如何与该细胞的IL-4受体结合, 如何触发细胞内的某种结构, 增加A-NK细胞的抗肿瘤作用的机制尚未知晓, 该项研究正在进行中.

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