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商陆多糖Ⅰ对小鼠腹腔巨噬细胞细胞毒作用及诱生肿瘤坏死因子和白细胞介素 1 的影响[1]

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Effects of *Phytolacca acinosa* polysaccharides Ⅰ on cytotoxicity of macrophages and its production of tumor necrosis factor and interleukin 1[1]

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ABSTRACT The *in vivo* effects of *Phytolacca acinosa* polysaccharides Ⅰ (PEP-Ⅰ) on immunologic cytotoxicity of mouse peritoneal macrophages and its production of tumor necrosis factor (TNF) and interleukin 1 (ⅠL-1) were studied. PEP-Ⅰ 80, 160 mg/kg was given ip twice every 4 d. Both doses were found to have significant enhancing activity on macrophages cytotoxicity against S180 sarcoma cells and malignant transformed fibroblast L929 cells. Peritoneal activated macrophages were incubated with LPS for 2 and 24 h to induce TNF and ⅠL-1, respectively. The TNF and ⅠL-1 activities were tested from cytotoxicity against L929 cells in an absorbence assay of enzymatic reaction and proliferation of thymocytes co-stimulated assay separately. The optimal time for TNF production was found on d 8. Singnificant increases in TNF and ⅠL-1 were observed. In comparison of the effect of PEP-Ⅰ on TNF with that of known priming agent BCG, there was no difference between these two, but PEP-Ⅰ had a high effect on ⅠL-1. These results suggest that cytotoxicity of macrophages primed by PEP-Ⅰ is closely related to its TNF and IL-1 production.

KEY WORDS *Phytolacca acinosa*; polysaccharides; macrophages; immunologic cytotoxicity; tumor necrosis factor; interleukin 1

提要 小鼠相隔 4 d ip 商陆多糖Ⅰ 80~160 mg/kg 二次可使腹腔巨噬细胞(MΦ)对 *S180* 和 *L929* 细胞的免疫细胞毒作用增强; 使脂多糖(LPS)辅助诱生肿瘤坏死因子(TNF)和白细胞介素 1(ⅠL-1)平行增加. 诱生 TNF 的达峰时间是二次 ip 后的 d 8. 与已知启动剂 BCG 相比,诱生 TNF 无差别, 诱生ⅠL-1 则比 BCG 高. 提示商陆多糖Ⅰ增强 MΦ 细胞毒作用与其诱生 TNF 和ⅠL-1 密切相关.

关键词 商陆; 多糖; 巨噬细胞; 免疫细胞毒性; 肿瘤坏死因子; 白细胞介素[1]

多糖抗瘤作用的机理认为可能与巨噬细胞(MΦ)参与的机体免疫功能提高有关[1]. 某些物质(如 BCG、酵母多糖等)在体内能激活 MΦ, 激活 MΦ 可通过释放一些细胞因子如肿瘤坏死因子(TNF)、白细胞介素 1 (IL-1)等来杀伤肿瘤细胞[2,3]. 商陆 (*Phytoclacca acinosa* Roxb)为传统的抗癌中药, 其中商陆多糖Ⅰ(PEP-Ⅰ)是其药用块根中含量较高的成分, 它能促进淋巴细胞增殖和产生白细胞介素 2 (ⅠL-2)以及增强 MΦ 的吞噬功能(中国药理通讯 1989; 6 : 22). 为进一步了解其作用机理, 本文探讨了 PEP-Ⅰ对小鼠腹腔 MΦ 细胞毒作用及诱生 TNF 和ⅠL-1 的影响

MATERIALS AND METHODS

PEP-Ⅰ是商陆药用块根水提液分离出来的一种酸性杂多糖, 内含半乳糖醛酸、半乳糖、阿拉伯糖和鼠李糖成分. 其分子比为 1:0.18:0.32:0.16, 分子质量为 10kDa. 临用时用生理盐水配成溶液, 水浴煮沸灭菌. 脂多糖(lipopolysaccharide, LPS)和放线菌素 D 为 Sigma 产品. 卡介苗(BCG)为上海生物制品所产品. [^{3}H]TdR 为中科院上海原子核研究所产品, 比活度为 481 GBq/mol. 重组人

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TNF(rHuTNF)为 Dainippon 公司 Sohmura 博士惠赠. 培养液为 RPMI-1640(Sigma), 每 1000 ml 补充 2-巯基乙醇 0.1 mmol, 青霉素 5000 IU, 链霉素 25mg, $NaHCO_3$ 2 g. 酶反应底物: 取 *p*-nitrophenol-*N*-acetyl-*D*-glucosaminide (NAG,上海医药工业研究院产品) 256.5mg 溶于 100ml(0.1 mol/L)枸橼酸缓冲液(pH 5.0), 加入等量的 0.5% Triton X-100 混匀, 过滤后分装, -18℃贮存. 酶反应终止剂: 取甘氨酸 0.75g, 氢氧化钠 0.309g, EDTA-Na_2 0.372g,加蒸馏水至 200 ml 即得.

腹腔 MΦ 的制备 ICR 小鼠,♀♂不拘, 21.4±SD 1.5g, 6～8 wk, 本校动物所提供. 相隔 4 d ip ×2 PEP-I, d 8 收集腹腔渗出细胞(PEC)用含 10%小牛血清的培养液将细胞悬浮, 取此细胞悬液适量加入大培养瓶中, 置 5%CO_2 孵箱中 37℃培养 2h 后, 弃去培养液, 再加入含 0.2% EDTA 的 PBS 溶液, 孵育 30 min, 用吸管吹落贴壁的 MΦ, 以 PBS 洗二次, 最后加入培养液使 MΦ 悬浮, 台盼兰染色, 计数.

巨噬细胞细胞毒试验 在 24 孔细胞培养板中每孔加入 MΦ 1×10^6/ml 和 S180 或 L929 肿瘤细胞各 100μl, 使效应细胞:靶细胞为 10:1 或 25:1, 同时设置肿瘤细胞加培养液的对照孔. 培养 48 h 后, 每孔加入[^{3}H]TdR 18.5 kBq, 继续培养 2 h, 收集细胞, 测定肿瘤细胞参入的 dpm 值, 按式+计算参入抑制率以反映细胞毒作用.

TNF 和 IL-1 的诱生及活性测定 在 24 孔细胞培养板中加入 PEC 3×10^6/ml, 置 5%CO_2 孵箱中培养 2h 后, 用 PBS 洗去未贴壁的细胞, 向贴壁的 MΦ 中加入含 LPS 10 μg/ml 的培养液 1 ml, 一部分样本继续培养 2h, 取上清供测定 TNF 用; 另一部分继续培养 24h, 取上清供测定 IL-1 用. 实验同时设置 BCG ip ×1 的阳性对照.

TNF 生物活性测定 用培养液将 L929 细胞浓度调整为 5×10^6/ml, 取此悬液 100μl 加入 96 孔细胞培养板中. 置 5% CO_2 孵箱中 37℃培养过夜, 倾去上清, 加入倍比稀释的待测 TNF 样品和放线菌素 D(终浓度 1μg/ml)各 100μl, 同时设 rHuTNF 标准对照. 继续培养 18h 后, 用 Landegren 酶反应微量比色法[4]测定吸收度(A)值. TNF 活性以样品杀伤 L929 细胞%表示.

IL-1 活性测定 用本室建立的方法[5].

RESULTS

PEP-I对腹腔 MΦ 细胞毒作用的影响 小鼠 ip ×2 PEP-I 后, 取其 MΦ, 观察 MΦ 对 S180 和 L929 肿瘤细胞的细胞毒性, 发现 PEP-I 处理鼠的 MΦ 明显抑制[^{3}H]TdR 的参入(Tab 1).

Tab 1. Effects of *Phytolacca acinosa* polysaccharides I (PEP-I) on the *in vitro* cytotoxicity of macrophages against S180 and L929 cells. Macrophages were prepared from ICR mice treated ip with two doses of PEP-I every 4 d on d 8. $n=3$, $\bar{x}\pm$ SD. $^{***}P<0.01$ *vs* control.

	PEP-I (mg/kg)	Cytotoxicity* at effector:target 10:1	25:1
S180	Saline	−4.9±2.3	
	80	23.3±3.5***	
	160	59.5±17.4***	
L929	Saline	29.1±4.4	53.4±5.3
	80	61.1±1.6***	75.5±2.3***
	160	56.2±1.6***	74.8±3.7***

* Cytotoxicity = (1 − dpm in treated sample / dpm in control) × 100%

PEP-I 对腹腔 MΦ 产生 TNF 和 IL-1 的影响 PEP-I 处理鼠的 MΦ 与 LPS 在体外共育后, 测定 MΦ 上清中 TNF 和 IL-1 含量, 发现 PEP-I 处理鼠的 MΦ 产生 TNF 和 IL-1 明显增加, 与已知启动剂 BCG 处理鼠相比, TNF 产生无明显差别($P>0.05$), IL-1 产生明显增高($P<0.05$)(Tab 2).

为观察 PEP-I影响 MΦ 产生 TNF 的动

Tab 2. Tumor necrosis factor (TNF) and interleukin 1 (IL-1) production in lipopolysaccharides (10μg / ml) supernatants of macrophages from mice pretreated ip with two doses of PEP-I every 4 d or a single dose of BCG. Cultures were set on d 8. Level of TNF was compared at 1 : 2 dilution ($n=3$) and expressed as cytotoxicity against L929 cells. Level of IL-1 was compared at 1 : 8 ($n=2$) and evaluated as [^{3}H] thymidine incorporation by thymocytes. $\bar{x}$ ± SD. $^{***}P<0.01$ vs control.

Sample	Dose (mg / kg)	TNF activity (%)	IL-1 activity (dpm)
Saline		−0.9 ± 3.2	870 ± 32
PEP-I	80	28.3 ± 5.0***	3736 ± 95***
	160	34.5 ± 0.6***	4302 ± 222***
BCG	300	39.2 ± 5.0***	2972 ± 137***

态变化，实验中 PEP-I 160 mg / kg × 2 ip，于不同时间处死小鼠，采集 PEC，制备 MΦ 后，诱生 TNF，发现 ip 后 d 6 培养上清中开始出现 TNF 活性，d 8 达到高峰，以后随着时间延长，TNF 活性逐渐下降(Tab 3).

Tab 3. Time course of TNF production in macrophages culture supernatant after PEP-I was injected ip 160 mg / kg twice every 4 d. TNF activity was measured at 1 : 2 dilution in an absorbence assay of enzymatic reaction. TNF activity of control was −0.9 ± 3.2, $n=3$, $\bar{x}$ ± SD. $^{*}P>0.05$ $^{***}P<0.01$ vs control.

Time (d)	TNF activity(%)
6	17.7 ± 2.6***
8	33.6 ± 8.9***
9	25.7 ± 1.8***
10	23.0 ± 2.6***
12	14.2 ± 1.8***
14	2.6 ± 8.8*

DISCUSSION

本实验表明 PEP-I 能显著增强 MΦ 对 S180 和 L929 肿瘤细胞的细胞毒作用，由于 TNF 单克隆或多克隆抗体可抑制 MΦ 杀瘤作用[6]，故 PEP-I 激活的 MΦ 上存在 TNF 可能是其增强 MΦ 细胞毒作用的重要机理之一.

已知 TNF 在体外能杀伤肿瘤细胞，在体内能导致肿瘤出血性坏死[7].IL-1 对某些肿瘤细胞也有抑制和杀伤作用[2].PEP-I 激活的 MΦ 在 LPS 辅助下释放 TNF 和 IL-1 增加，表明 PEP-I 激活的 MΦ 尚有潜在的抗肿瘤作用.因为 IL-1 能与抗原或丝裂原协同激活 T_H 细胞产生 IL-2[8]，所以 PEP-I 增强 MΦ 产生 IL-1 可能是其促进淋巴细胞增殖和产生 IL-2 的原因之一.

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