Effect of *Phytolacca acinosa* polysaccharides I on production of colony-stimulating factors of mouse splenocytes in vitro

WANG Hong-Bin, ZHENG Qin-Yue, CHEN Hai-Sheng
department of Pharmacology, Department of Phycology, College of Pharmacology, Second Military Medical University, Shanghai 200433, China

ABSTRACT Phytolacca acinosa polysaccharides I (PAP-1), $M_r = 10,000$, activated mouse splenocytes to produce colony-stimulating factors (CSF) in vitro. The level of CSF was tested by $[^3H]Tdr$ uptake by bone marrow cells and rmGM-CSF was used as standard. PAP-1 (10–500 μg·mL$^{-1}$) increased CSF production of the splenocytes treated with or without concanavalin A. When the concentration of PAP-1 was 100 μg·mL$^{-1}$, the level of CSF was about equivalent to that of rmGM-CSF 11.8 ± 1.8 ng·mL$^{-1}$. After a 3-day incubation of PAP-1 with the splenocytes, CSF was assayed. The longer the incubation, the higher were the CSF concentrations. The CSF type in supernatants of splenocytes induced by PAP-1 was determined by IL-3 McAb, GM-CSF McAb, and M-CSF PeAb.

Received 1991-07-09 Accepted 1993-01-10

1. Project supported by the Science Foundation of the General Logistics Department of the PLA, No. 26-071-176.

The type of CSF was found to be interleukin-3.

KEY WORDS Phytolacca acinosa; polysaccharides; concanavalin A; colony-stimulating factors; interleukin-3; spleen
**RESULTS**

PAP-I 体外对脾细胞产生 CSF 的影响

PAP-I (10～1000 μg·mL⁻¹) 和小鼠脾细胞共同培养3 d，发现 PAP-I 可显著促进脾细胞产生 CSF，PAP-I 100 μg·mL⁻¹ 可刺激产生相当于 rmGM-CSF 11.8±1.8 ng·mL⁻¹ 的 CSF (Tab. 1)。Con A 0, 1, 2.5, 5, 10, 50 μg·mL⁻¹ 刺激小鼠脾细胞产生 CSF 含量分别相当于 rmGM-CSF 7.7, 9.9, 11.5, 13.3, 12.1 和 7.3 ng·mL⁻¹，说明 Con A 刺激 CSF 产生的最佳刺激浓度为 5 μg·mL⁻¹。选择 Con A 1, 2.5, 5 μg·mL⁻¹ 和 PAP-I 共同培养 3 d，发现 PAP-I 可显著促进 Con A 1, 2.5 μg·mL⁻¹ 诱导的脾细胞产生 CSF，而 Con A 浓度为 5 μg·mL⁻¹ 时，加 PAP-I 与单独 Con A 之间无显著性差异 (Tab. 1)。

<table>
<thead>
<tr>
<th>Tab. 1</th>
<th>Colony stimulating factors (CSF) production (ng·mL⁻¹) in supernatants of Con A-induced mouse splenocytes treated with Phytolacca acinosa polysaccharides I (PAP-I) for 3 d. CSF was measured by radio-activity of [³H]TdR uptake by bone marrow cells and rmGM-CSF was used as standard. n = 4 experiments, each experiment pooled from spleens of 3-4 BALB/c mice. *P &lt; 0.01 vs control.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP-I/</td>
<td>Concanaeatin A/μg·mL⁻¹</td>
</tr>
<tr>
<td>mg·mL⁻¹</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.7±0.6</td>
</tr>
<tr>
<td>10</td>
<td>10.2±1.7</td>
</tr>
<tr>
<td>100</td>
<td>11.8±1.8</td>
</tr>
<tr>
<td>200</td>
<td>10.2±0.7</td>
</tr>
<tr>
<td>500</td>
<td>10.9±1.5</td>
</tr>
<tr>
<td>1000</td>
<td>8.4±2.1</td>
</tr>
</tbody>
</table>

**PAP-I 体外刺激脾细胞产生 CSF 时效关系**

PAP-I 和小鼠脾细胞共同培养不同时间，收
集上清液检测CSF，发现PAP-1和单克隆抗体
3d上清液中开始出现CSF，并随着培养时间
的延长，CSF含量逐渐升高（Fig 1）。Con A
刺激脾细胞产生的CSF含量随着时间延长
越来越高。至3d达峰，以后逐渐下降，
PAP-1合用Con A培养至7d，其刺激产生的
CSF含量显著地高于单用Con A组（Fig 1）。

(P < 0.001)，而与仅为培养基（不含检测上
清）的阴性对照孔dpm值993±110无显著差
异，说明PAP-1刺激脾细胞产生的CSF类型
主要为 interleukin-3。

Tab 2. CSF types from mouse splenocyte supernatants
   treated with PAP-1. CSF was measured by D-[3H]
galactose uptake by membrane of bone marrow cells.
n = 3 - 4 BALB c mice in each experiment. * P > 0.05,
   ** P < 0.01 vs + RPMI-1640.

<table>
<thead>
<tr>
<th>Supernats of PAP-1</th>
<th>CSF activity/dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>+GM-CSF, McAb</td>
<td>1.283±60*</td>
</tr>
<tr>
<td>+M-CSF, PcAb</td>
<td>1.253±68*</td>
</tr>
<tr>
<td>+IL-3, McAb</td>
<td>865±73*</td>
</tr>
<tr>
<td>+RPMI-1640</td>
<td>1.375±88</td>
</tr>
<tr>
<td>RPMI-1640 only</td>
<td>993±110*</td>
</tr>
</tbody>
</table>

**DISCUSSION**

本文实验结果证明了PAP-1能显著促进
脾细胞产生CSF。经IL-3、GM-CSF单克隆
抗体及M-CSF的多克隆抗体吸收实验证实
CSF类型主要为IL-3。已知激活T淋巴细胞
可产生IL-3，推测PAP-1可能通过激活T
淋巴细胞产生IL-3，此和PAP-1能激活T淋
巴细胞产生IL-2的报道一致（中国药理通讯
1989；6：22）。PAP-1激活T淋巴细胞可能
是直接或间接的。本文实验结果表明PAP-1
可以显著促进亚致剂量Con A刺激小鼠脾细
胞产生CSF，而Con A浓度达最适刺激浓度5
µg·ml⁻¹时，PAP-1合用Con A与单独使用
Con A刺激小鼠脾细胞产生CSF能力无显著
差异，此结果说明PAP-1与Con A刺激CSF
产生的靶细胞可能是一致的。欲进一步区分
PAP-1作用的靶细胞类型，还有待将脾细胞
PAP-1 对其产生 CSF 的影响。已知实验表明 IL-3 能诱导单核-巨噬细胞与 TNF 合成有关的 mRNA，PAP-1 可促进 Mφ 分泌 IL-1 并可作为启动剂促进 TNF 分泌，说明 PAP-1 促进 T 淋巴细胞产生 IL-3 可能是其启动 Mφ 分泌 TNF 的重要机制之一。本实验结果以及以往实验提示 PAP-1 可能具有活化启动及造血功能的保护作用。

REFERENCES


Papers are welcome

Acta Pharmacologica Sinica publishes papers of a broad range of topics of biomedical sciences, both experimental and clinical. Manuscripts in English of original research from any country, are welcome.

Acta Pharmacologica Sinica is published bimonthly and listed in Abstracts of Chinese Medicines, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents/Life Sciences, de Haen's Drugs in Prospect, Excerpta Medica, Index Medicus, Research Alert, Science Citation Index, SciSearch, Tropical Diseases Bulletin, Референтный Журнал, etc.


An ABSTRACT (no more than 150 words) is followed by 3-10 KEY WORDS, using terms from the medical subject headings (MeSH) list of Index Medicus when possible. Mean values must be accompanied by s (SD, not SEM). Body weights are expressed in actually measured ±. Do not include more significant digits in the data than are justified by the accuracy of the determinations. Use the Système International d'Unités (SI units). The statistical significances are indicated by: *P > 0.05, **P < 0.05, ***P < 0.01. The number of REFERENCES should not exceed 15.