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# Influences of Polyactin A on activities of human monocytes *in vitro*

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**ABSTRACT** Effects of Polyactin A (PAA) on abilities of human monocytes to synthesize and secrete interleukin-1 (IL-1) and to modulate natural killer (NK) cell activity in large granular lymphocytes (LGL) were investigated *in vitro*. Over a wide range of concentrations (0.01-100 $\mu g \cdot ml^{-1}$), PAA directly induced IL-1 synthesis and secretion, showing the maximal effect at 10 $\mu g \cdot ml^{-1}$, and evidently synergized with lipopolysaccharides (LPS) of *E coli* in stimulation of IL-1 production by human monocytes. The manifestation of PAA pretreated autologous monocytes in modulation of NK cell activity was closely related to PAA concentration. A boosting effect of PAA-treated monocytes on NK activity was observed when PAA 10-100 $\mu g \cdot ml^{-1}$ were used for pretreatment of monocytes, while an inhibitory influence of monocytes was found when PAA 0.01-0.1 $\mu g \cdot ml^{-1}$ were used. These results demonstrate significant effects of PAA on functions of human monocytes, enhancing IL-1 production and affecting their regulative activity on NK cell cytotoxicity.

**KEY WORDS** Polyactin A; monocytes; interleukin-1; natural killer cells

The novel immunopotentiator Polyactin A (PAA), developed in China, is a hetero-polysaccharide isolated from fermentation broth of buccal alpha-hemolytic streptococci strain No 33. In the past few years, nonspecific stimulating effects of PAA on host immunity were reported. The promoting effects of PAA on antitumor immunity included promotion of phagocyte activity[1], stimulation of proliferation of thymic and spleen lymphocytes[2,3], enhancement of lymphocyte transformation[4], increase in antibody production[5], augmentation of quantity of serum complement[6], and induction of interleukin-1 (IL-1)[7] and interleukin-2 (IL-2) production[8].

So far, only its influence on phagocytosis of murine macrophages was reported. The present communication described the modulating effects of PAA on IL-1 production and regulation of NK cell activity by human peripheral blood monocytes.

## MATERIALS AND METHODS

**Reagents** PAA was produced by Lihua Pharmaceutical Factory, Chengdu (Lot 880320). RPMI 1640 medium was purchased from Gibco Lab, USA; complete RPMI 1640 medium (CM) was prepared as described previously[8]. LPS (*E coli* 055 : B5) was purchased from Difco, USA; concanavalin A (Con A) from Sigma, USA; Percoll from Pharmacia Chemicals, Sweden. All other chemicals were of the highest grade commercially available.

**Animals and cell line** Male C57BL/6J mice (6-8 wk of age) were provided by Sichuan Industrial Institute of Antibiotics, Chengdu. Human erythroleukemic cell line K562 was purchased from Chongqing Medical University, Chongqing, and grown as suspension culture.

**Human blood samples** The heparinized venous blood samples were purchased from Chengdu Central Blood Bank. The blood

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donors of both sexes were healthy, 23–30 a old, took no medications, and fasted for 12 h before venipuncture.

**Separation of monocytes** Mononuclear cells (MNC) were isolated immediately from the blood samples by Ficoll–Hipaque density centrifugation. Adherent monocytes were then separated with new born calf serum–precoated plastic Petri dishes (Falcon, USA)[9].

**Purification of LGL** LGL were obtained by centrifugation on a discontinuous density gradient of Percoll. CM and Percoll were adjusted to 285 mOsmol / kg $H_2O$ with sterile distilled water and 10× concentrated PBS, respecteively. Seven different concentrations of Percoll in CM were prepared, ranging from 40% to 57% Percoll, and layered into test tubes. Monocyte–depleted MNC $5\times10^7$ were placed on the top of the gradient, and the tubes were spun at $550\times g$ for 30 min. Cells from the top 3 interfaces were collected, pooled, washed, and resuspended in CM.

**IL–1 production** Monocytes were placed in 24–well plates (Linbro, USA) at $1\times10^6$ / well in 1 ml CM. IL–1 production was initiated by the addition of PAA at various concentrations, LPS (10 $\mu g\cdot ml^{-1}$) or both at 37℃ in air of 5% $CO_2$ for 24 h. Control wells contained cells without PAA or LPS. After incubation, PAA 100 $\mu g\cdot ml^{-1}$, LPS 10 $\mu g\cdot ml^{-1}$ or both were added to the control wells, then the supernatants containing extracellular IL–1 were collected from all wells and clarified by centrifugation. The remaining adherent cells were covered with 1 ml fresh CM, disrupted by repeated freezing and thawing in a methanol bath, and sonicating. The supernatants containing intracellular IL–1 were obtained by centrifugation. All the supernatants were dialysed against PBS at 4℃ for 24 h, passed through a millipore filter (0.22 $\mu m$) and stored at −70℃ until tested for IL–1 activity.

**IL–1 measurement** Thymocytes ($1.5\times10^6$ / well) isolated from C57BL / 6J mice were co–cultured, in triplicate wells of 96–well plates (Costar, USA), with the monocyte culture supernatant in the presence of Con A at a final concentration of 1 $\mu g\cdot ml^{-1}$. The cultures were incubated for 72 h, pulsed with 46.25 kBq / well of [$^3$H]TdR for the final 16 h and harvested onto glass filter strips, and the radioactivity was determined in a LS 9800 liquid scintillation counter (Beckman, USA). IL–1 activity was evaluated by [$^3$H]TdR incorporation into the murine thymocytes.

**IL–2 bioassay** IL–2 activity was measured as the ability to support IL–2–dependent cytotoxic T–cell line proliferation[8].

**NK cytotoxicity measurement** NK cell activity was measured by the DNA–synthesis of target K562 cells[10]. The % of specific inhibition (PI) of [$^3$H]TdR incorporation into the target cells was calculated by the following formula for triplicate samples:

PI = [1−(Test incorporation − Effector spontaneous incorporation) / Target spontaneous incorporation] × 100%

**Statistical analysis** Each experiment was repeated 3–5 times. Data are given as $\bar{x}\pm s$ or the representative results of the separate determinations. The differences between pairs of measurements from the same normal individuals were examined by paired $t$ test.

## RESULTS

**Inducing effect of PAA on IL–1 secretion** Monocytes were incubated in the presence of PAA 10 $\mu g\cdot ml^{-1}$ for 24 h, and IL–1 activity was assayed in the cultural supernatants. PAA significantly induced IL–1 release from human monocytes (Tab 1).

IL–2 capable of causing murine thymocytes to proliferate may interfere with IL–1 assay in our IL–1 assay system. IL–2 activity, however, was not detected in all the

**Tab 1. IL-1 secretion from human monocytes by PAA 10 $\mu g \cdot ml^{-1}$. IL-1 activity is expressed as [$^3$H]TdR uptake by murine thymocytes (dpm). $n=3$, $\bar{x} \pm s$. $^{***}P<0.01$ *vs* control.**

| Expt | Control monocytes | PAA-treated monocytes |
|---|---|---|
| 1 | 1 511 ± 628 | 6 370 ± 720*** |
| 2 | 2 040 ± 774 | 5 487 ± 1 126*** |
| 3 | 2 800 ± 1 038 | 13 731 ± 971*** |
| 4 | 2 278 ± 733 | 11 744 ± 1 895*** |
| 5 | 1 870 ± 610 | 10 408 ± 924*** |

supernatants of monocyte cultures without or with PAA (dpm < 450).

**Kinetics of IL-1 release from monocytes treated with PAA** After co-culture of human monocytes with PAA 10 $\mu g \cdot ml^{-1}$ for 0–48 h, the cultural supernatants were tested for IL-1 activity. Augmentation of IL-1 level induced by PAA was seen as early as 2 h of incubation; IL-1 release peaked at 24 h and then was on the wane (Tab 2).

**Tab 2. IL-1 release from human monocytes cocultured with PAA. IL-1 activity is given as [$^3$H]TdR incorporation into mouse thymocytes (dpm). $n=4$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. Similar results were obtained in 3 separate experiments.**

| Incubation time, h | [$^3$H]TdR incorporation, dpm Medium | PAA 10 $\mu g \cdot ml^{-1}$ |
|---|---|---|
| 0 | 771 ± 287 | 904 ± 509* |
| 2 | 1 137 ± 711 | 2 722 ± 1 105** |
| 10 | 1 462 ± 464 | 6 442 ± 839*** |
| 16 | 1 973 ± 655 | 10 399 ± 962*** |
| 24 | 2 659 ± 213 | 13 592 ± 1 184*** |
| 36 | 1 406 ± 832 | 8 238 ± 2 520** |
| 48 | 935 ± 489 | 4 215 ± 735*** |

**PAA concentration-dependent enhancement of IL-1 production** Human monocytes were incubated for 24 h in the presence of PAA 0–100 $\mu g \cdot ml^{-1}$, and extra- and intracellular IL-1 activities of the monocytes were determined. Tab 3 clearly showed the concentration-dependent enhancing effects of PAA on IL-1 production. As little as PAA 0.01 $\mu g \cdot ml^{-1}$ exhibited significant enhancing effects, and the maximal effects were observed when PAA was 10 $\mu g \cdot ml^{-1}$.

**Tab 3. Effects of PAA on IL-1 synthesis and seretion from monocytes. IL-1 activity is shown as [$^3$H]TdR uptake by mouse thymocytes. $n=3$, $\bar{x} \pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. Similar results were obtained in 4 tests.**

| PAA, $\mu g \cdot ml^{-1}$ | LPS, $\mu g \cdot ml^{-1}$ | [$^3$H]TdR uptake, dpm Extracellular | Intracellualr |
|---|---|---|---|
| 0 | 0 | 926 ± 170 | 2 112 ± 520 |
| 0.01 | 0 | 2 843 ± 291** | 3 964 ± 614** |
| 0.1 | 0 | 5 345 ± 352*** | 5 771 ± 291*** |
| 1 | 0 | 9 363 ± 1 511*** | 8 435 ± 1 036*** |
| 10 | 0 | 13 049 ± 733*** | 8 850 ± 836*** |
| 100 | 0 | 10 935 ± 1 038*** | 7 570 ± 693*** |
| 0 | 10 | 8 435 ± 1 038 | 12 271 ± 783 |
| 0.01 | 10 | 12 632 ± 913** | 20 274 ± 1 713** |
| 0.1 | 10 | 19 601 ± 1 509*** | 29 619 ± 3 379*** |
| 1 | 10 | 24 287 ± 1 868*** | 31 565 ± 4 513*** |
| 10 | 10 | 28 437 ± 2 121*** | 41 126 ± 5 099*** |
| 100 | 10 | 26 372 ± 2 975*** | 40 081 ± 4 108*** |

**Synergic stimulation of IL-1 production by PAA and LPS** LPS is a well known stimulant of IL-1 production, thus it was of interest to determine whether PAA would synergize with LPS in the IL-1 production by human monocytes. Results shown in Tab 3 provide evidence confirming this synergy of PAA and LPS in IL-1 production.

**Augmentation of intracellular IL-1 by PAA** Since IL-1 synthesis and secretion by human monocytes may well be two distinct biological events[12], we also observed effect of PAA on intracellular IL-1. Tab 4 demonstrates that either in the presence or absence of LPS 10 $\mu g \cdot ml^{-1}$, PAA (10 $\mu g \cdot ml^{-1}$) resulted in marked elevation of not only extracellular but also intracellular IL-1, and that PAA alone exhibited stronger stimulatory effect on IL-1 secretion than that on IL-1

**Tab 4. IL-1 synthesis and secretion from human monocytes treated with PAA 10 μg · ml$^{-1}$, LPS 10 μg · ml$^{-1}$ or both. IL-1 activity is presented as [$^3$H]TdR incorporation into murine thymocytes. $n=5$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$ *vs* control.**

| Stimulus added | [$^3$H]TdR incorporation, dpm Extracellular | Intracellualr |
|---|---|---|
| None | 2 087 ± 500 | 3 749 ± 688 |
| PAA | 9 540 ± 3 531 | 9 895 ± 1 067 |
| LPS | 8 009 ± 917 | 13 529 ± 1 179 |
| PAA + LPS | 25 195 ± 4 785 | 41 666 ± 3 753 |
| Ratio PAA / None | 4.57 | 2.64** |
| Ratio (PAA + LPS)/LPS | 3.15 | 3.08* |

synthesis.

**Modulation of NK activity by autologous monocytes pretreated with PAA** PAA showed boosting effect on NK activity in highly purified human LGL[11]. To understand role of PAA-treated monocytes in NK cell activity, isolated autologous monocytes were incubated without or with PAA 0-100 μg · ml$^{-1}$ for 24 h, washed, added in different proportions (0%-10%) to LGL, and then NK activity was measured. Effect of PAA-pretreated autologous monocytes on NK cell activity was closely dependent on PAA concentration used. PAA (0.01-0.1 μg · ml$^{-1}$)-treated autologous monocytes suppressed NK activity; the cells precultured with PAA 1 μg · ml$^{-1}$ had no siginificant influence on NK activity; while those cells preincubated in PAA 10-100 μg · ml$^{-1}$ remarkably augmented NK activity (Tab 5).

**Tab 5. NK activity of human LGL in the presence of added autologous monocytes precultured with PAA for 24 h and washed. NK activity is expressed as % of inhibition (percentage of enhancement) of [$^3$H]TdR incorporation into target K562 cells by effector LGL at E / T ratio of 10 : 1. $n=4$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. [a] Percentage of monocytes added to LGL cultures before assay.**

| PAA, μg · ml$^{-1}$ | % of inhibition of [$^3$H]TdR incorporation into K562 cells 0%[a] | 5%[a] | 10%[a] |
|---|---|---|---|
| 0 | 25.5 ± 5.2 | 23.4 ± 4.7* (−7.1) | 20.8 ± 5.4* (−17.5) |
| 0.01 | | 15.6 ± 2.8** (−38.1) | 8.4 ± 3.2** (−66.7) |
| 0.1 | | 18.3 ± 3.6** (−27.4) | 13.6 ± 2.5** (−46.0) |
| 1 | | 24.9 ± 4.4* (−1.2) | 22.7 ± 4.2* (−10.7) |
| 10 | | 31.7 ± 6.4** (+25.8) | 29.4 ± 6.7* (+16.7) |
| 100 | | 37.3 ± 5.2*** (+48.0) | 44.6 ± 8.1*** (+81.7) |

## DISCUSSION

The results of the present study suggest that the boosting effect of PAA on IL-1 synthesis and secretion from human monocytes might, in part, mediate the antitumor activity of this novel compound.

It is well established that IL-1 mediate a large number of diverse immunostimulatory events, including stimulation of T-cell proliferation, co-stimulation of B-cells, regulation of B-cell differentiation and acting on lymphocytes to promote generation of IL-2 which is a crucial mediator in cellular immunity. Because of the key role played by IL-1 in generation of an immune response, our findings not only provided an explanation for the mechanisms of action of PAA in treatment of tumors, but also suggested an possible application of this novel immunopotentiating agent in a wider range.

The results indicated that the augmenting effects of PAA on IL-1 synthesis and release from human monocytes *in vitro* were concentration-dependent, and this manner of action was different from that observed in the experiments using murine peritoneal macrophages[11]. Although the stimulatory effects of PAA on IL-1 synthesis and secretion

occur independently of the stimulation of monocytes observed with LPS, the mechanism by which PAA boosts IL-1 production from human monocytes is not clarified in the present investigation and awaits further studies.

Human monocytes have been reported to exhibit both spontaneous up-regulation and suppression of NK cell activity. From our study, it is clear, that neither PAA-treated nor untreated autologous monocytes were directly cytotoxic to K562 cells *in vitro*, but they did affect NK activity of human LGL, confirming their regulating effect on NK activity. There are observations reported that macrophages are not involed in the *in vitro* enhancement of NK activity in mice, while other reports demonstrate that macrophages are required for the *in vivo* augmentation of NK activity by Bacillus Calmette Guerin. The present data indicate that the modulating effect of PAA-treated autologous monocytes on NK activity of human LGL was closely related to the concentration of PAA used. Exogenous agents capable of modulating NK cell and monocyte responses are of interest because of their potential usefulness in elucidating the mechanism of interactions between these two cell populations and in achieving an effective augmentation of natural immune responses. In this aspect, PAA seems to fit into this category of agents. Our observation demonstrates that a relatively large concentration of PAA is required to achieve boosting effects of monocytes on human NK activity *in vitro*. If this *in vitro* finding can be confirmed *in vivo*, it will be practically helpful in clinical determination of suitable PAA dose.

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## 多抗甲素体外对人单核细胞功能的影响

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**提要**　本文探讨多抗甲素体外对人单核细胞生成 IL-1 及调节 NK 细胞活性的功能的影响．多抗甲素 0.01-100 μg · ml$^{-1}$ 既能直接诱导人单核细胞生成 IL-1，也能与大肠杆菌脂多糖协同作用刺激 IL-1 生成．多抗甲素诱导单核细胞分泌 IL-1 比合成 IL-1 的作用强、影响单核细胞调节 NK 细胞活性的作用与其浓度密切相关、较低浓度多抗甲素预处理后、单核细胞抑制 NK 活性、高浓度多抗甲素处理后，单核细胞则促进 NK 活性．

**关键词**　多抗甲素；单核细胞；白细胞介素-1；自然杀伤细胞

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# Effect of tetrandrine on myocardial $Na^+$, $K^+$-ATPase in renovascular hypertensive rats

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**ABSTRACT**　In renovascular hypertensive rats (RVHR, two-kidney, one-clip model), the myocardial $Na^+$, $K^+$-ATPase showed a reduced activity. Though its sensitivities to $K^+$ and ouabain (Oua) were not changed, the enzyme was less responsive to $Na^+$ and ATP, and also much more susceptible to the inhibitory effect of $Ca^{2+}$. Tetrandrine (Tet, ig 50 mg · kg$^{-1}$, qd × 26 d) increased the myocardial $Na^+$, $K^+$-ATPase activity in RVHR. However, *in vitro*, Tet elevated moderately the enzyme activity in RVHR only at high concentrations (100 -1 000 μmol · L$^{-1}$ ), and failed to influence the enzyme activity in normotensive rats. In RVHR, treatrment by Tet *in vivo* increased the degree of the $Na^+$, $K^+$-ATPase activation under suboptimal substrate ($Na^+$, $K^+$, or ATP) concentrations and antagonized the inhibitory effect of $Ca^{2+}$ or Oua. Similar results were found when the enzyme preparation from RVHR was incubated with Tet 10 μmol · L$^{-1}$ during ATPase analysis. On the contrary, the myocardial $Mg^{2+}$-ATPase activity was higher in RVHR. Tet depressed this enzyme both *in vivo* and *in vitro*. These facts indicate that the increased myocardial $Na^+$, $K^+$-ATPase activity in RVHR is not only secondary to the calcium channel blocking or antihypertensive action of Tet but also due to its direct effects on the $Na^+$, $K^+$-ATPase and $Mg^{2+}$-ATPase.

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**KEY WORDS**　tetrandrine; renovascular hypertension; sodium, potassium adenosine triphosphatase; calcium; ouabain

The abnormalities of myocardial $Na^+$, $K^+$-ATPase, which have been observed in various forms of experimental hypertension[1-3], might be related to the hypertension and its subsequent heart complications. Our previous studies showed that tetrandrine (Tet) could improve the partially depressed myocardial $Na^+$, $K^+$-ATPase activity in normotensive rats (data to be published). However, it is yet unknown how the drug, as an antihypertensive agent, would influence the myocardial $Na^+$, $K^+$-ATPase activity in hypertensive rats. This study is to investigate what changes take place in the myocardial $Na^+$, $K^+$-ATPase isolated from the renovascular hypertensive rats (RVHR, two-kidney, one-clip model), and also to observe the effects of Tet on this enzyme both *in vivo* and *in vitro*.

## MATERIALS AND METHODS

**Reagents**　Tet was purchased from Jinghua Pharmaceutical Co. Ouabain (Oua)