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Endogenous pyrogen formation by human blood monocytes stimulated by polyriboinosinic acid:polyribocytidylic acid

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Abstract. The pyrogenic response to supernatants from human blood monocytes stimulated with polyriboinosinic acid:polyribocytidylic acid (poly I:C) was characteristic of a response to endogenous pyrogen in that it was brief and monophasic, and was destroyed by heating the supernatants at 70 °C for 30 min. Pyrogen production was unimpaired when the incubations were carried out in the presence of cycloheximide (50 μg/ml; an inhibitor of protein synthesis) or indomethacin (50 μg/ml; an inhibitor of prostaglandin synthesis). Also, neither interferon, interleukins, tumor necrosis factor nor prostaglandin E_2 were detectable in the supernatants from the poly I:C-stimulated human monocytes.
Key words. Monocytes; fever; endogenous pyrogen; polyriboinosinic acid:polyribocytidylic acid.

The first evidence for an endogenous pyrogen derived from a body tissue was provided by Beeson[1] and Bennett and Beeson[2,3], who extracted a pyrogenic substance from rabbit polymorph leukocytes and showed that the characteristics of this pyrogen differed from those of an exogenous pyrogen in several aspects. In 1967, monocytes and various tissues were also shown to produce endogenous pyrogens[4-6]. Our recent results showed that intravenous administration of polyriboinosinic acid:polyribocytidylic acid (poly I:C) produced febrile responses in rabbits, whereas poly I or poly C alone did not[7,8]. However, it was not known whether the fever induced by poly I:C was due to stimulation of endogenous release of pyrogens by the peripheral blood monocytes. Therefore, in the current experiments, the pyrogenic response to supernatants from the poly I:C-stimulated human blood monocytes was assessed in rabbits. In addition, the production of interferon, prostaglandin E_2 or other cytokines in the supernatants from the poly I:C-treated human blood monocytes was investigated.

Materials and methods

Human blood monocytes were obtained as the buffy coat fraction from healthy donors at the Tainan Blood Bank Center (Tainan City, Taiwan, ROC). They were isolated by centrifugation over a Ficoll-Paque (LKB Biotechnology) density gradient[9,10]. The cells collected at the interface were washed twice with serum-free RPMI-1640 (Gibco, Grand Island, N.Y.) and were subsequently resuspended in serum-free RPMI-1640 medium containing 2 mM L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. Then the human blood monocytes were incubated with poly I:C (Pharmacia Molecular Biologicals, Uppsala, Sweden), or combined with either indomethacin (50 μg/ml; Sigma) or cycloheximide (50 μg/ml; Sigma) at 37 °C in a shaker bath (220 rpm). After 1-h incubation, the cell suspensions were washed free of poly I:C and re-fed with fresh medium. The supernatants were harvested by centrifugation (1000 rpm, 10 min) and were stored at −80 °C until used for experiments or assays. All supernatants were screened for endotoxin contamination and found negative. All drug solutions were prepared in pyrogen-free glassware and containers. All solutions were passed through 0.22-μm Millipore bacterial filters. New Zealand-derived albino male rabbits weighing 2.5–3.9 kg were obtained from a single supplier. They were housed and trained for pyrogen testing as previously described[7,8]. A hypothalamic cannula (0.81 mm o.d.) was implanted into the preoptic anterior hypothalamus of each animal under general anesthesia (pentobarbital sodium, 30 μm/kg, i.v.). The procedures for cannulation and intrahypothalamic injections followed a published protocol[7]. A period of 2 weeks was permitted to allow the animals to recover before they received injections. The injection volume was 1.0 μl for all intrahypothalamic injections. At the end of the experimental series, the location of the intrahypothalamic cannula was confirmed as previously described[7].

Interferon activity titers were assayed by the cytopathic effect inhibition microassay method with human FL cells and vesicular stomatitis virus (VSV, Indiana strain) as the challenge virus[11-13]. The highest dilution of the titrated sample giving 50% protection was considered as the end-point. Samples were compared to a NIH reference human interferon-alpha standard (Ga 23-902-530). Interleukin-1-beta activity in the supernatants was determined using an ELISA kit from Cistron Biotechnology (Pine Brook, NJ), interleukin-6 using an ELISA kit

from R & D systems (Minneapolis, MN), and interleukin-1-alpha and tumor necrosis factor using kits from Endogen Inc. (Boston, MA). Prostaglandin E_2 (PGE_2) was determined using a specific RIA kit (NEN Research Products, Wilmington, DE) as detailed previously[8].

Results and discussion

Rabbits were given intravenous bolus injections of the supernatants (at a dose of ml/kg) obtained from various amounts of human blood monocytes incubated with poly I:C (100 µg/ml). The data are depicted in figure 1. It can be seen from the figure that intravenous administration of the supernatants from poly I:C (100 µg/ml)-treated monocytes using 1×10^5 cells/ml did not produce significant fever (<0.3 °C). However, administration of the supernatants from poly I:C (100 µg/ml)-treated monocytes using $3 \times 10^6 - 1 \times 10^8$ cells/ml produced dose-dependent fever in rabbits. The rectal temperature started to rise at 5–10 min after injection. The monophasic fever reached its peak at 150 min and returned to its preinjection level 6 h later. In addition, microinjection of the supernatants (at a dose of 1 µl per brain) obtained from the poly I:C (100 µg/ml)-treated monocytes (3×10^6 cells/ml) into the preoptic anterior hypothalamus also produced dose-dependent fever in rabbits (as shown in fig. 2). As compared to the undiluted supernatants, diluted supernatants (e.g. 2×, 5× or 10×) produced less fever in rabbits. Also, figure 3 shows that the supernatants (at a dose of 1 µl per animal) obtained from the poly I:C (0.8–100 µg/ml)-treated monocytes (3×10^6 cells/ml) produced dose-dependent fever in rabbits.

To test for the ability of poly I:C to stimulate the release of intermediates such as PGE_2, protein factors or thermal labile factors, pyrogen testing was carried out in rabbits treated with the supernatants from monocytes (3×10^6 cells/ml) + poly I:C (100 µg/ml), monocytes (3×10^6 cells/ml) + poly I:C (100 µg/ml) + indomethacin (50 µg/ml), or monocytes (3×10^6 cells/ml) + poly I:C (100 µg/ml) + cycloheximide (50 µg/ml). As shown in figure 4, indomethacin or cycloheximide pretreatment did not alter the pyrogenicity of the supernatants of poly I:C-treated monocytes. On the other hand, as compared to supernatants produced by poly I:C-treated monocytes at 37 °C, those heated at 70 °C for 30 min produced a lower fever when injected into the hypothalamus (fig. 5).

To test for the ability of poly I:C to stimulate the release of interferon, interleukins, tumor necrosis factor or

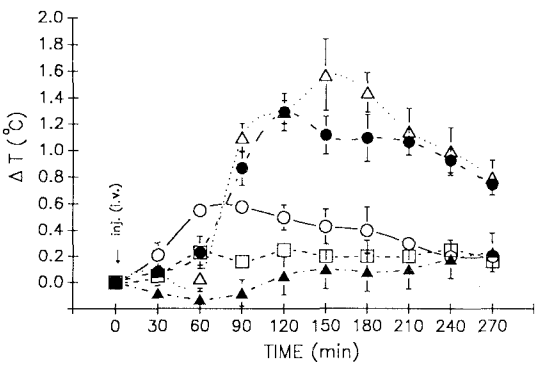

Figure 1. The mean (±SEM) change in rectal temperature of rabbits (N = 5 per group) injected intravenously (inj. i.v.) with normal saline or supernatants from poly I:C (100 µg/ml)-treated monocytes ($1 \times 10^5 - 1 \pm 10^8$ cells/ml). *Significantly different from corresponding control values (saline group), $p < 0.05$ (Student's t-test. ○—○: 1×10^8 cells/ml; ●--●: 1×10^7 cells/ml; △ ··· △: 3×10^6 cells/ml; ▲-·-▲: 1×10^5 cells/ml; □--□: saline.

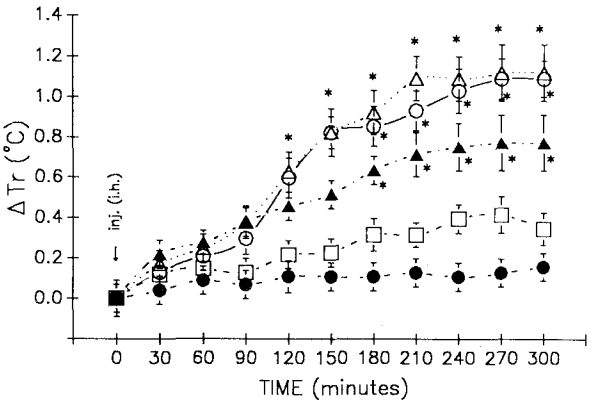

Figure 2. The mean (±SEM) change in rectal temperature of rabbits injected intrahypothalamically (inj. i.h.) with medium control or supernatants from poly I:C (100 µg/ml)-treated monocytes (3×10^6 cells/ml). *Significantly different from corresponding control values (medium control), $p < 0.05$ (Student's t-test). ○—○: Undilute (N = 6); △ ··· △: 2× dilute 2 µl (N = 6); ▲-·-▲: 5× dilute 2 µl (N = 6); □--□: 10× dilute 2 µl (N = 6); ●--●: medium control (N = 7).

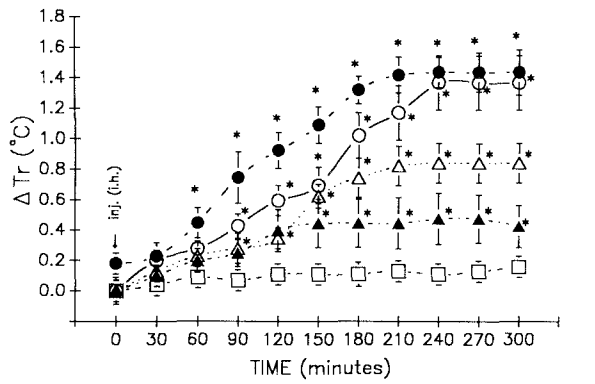

Figure 3. The mean (±SEM) change in rectal temperature of rabbits injected intrahypothalamically (inj. i.h.) with medium control or supernatants from poly I:C (0.8–100 µg/ml)-treated monocytes (3×10^6 cells/ml). *Significantly different from corresponding control values (medium control), $p < 0.05$ (Student's t-test). ○—○: Poly I:C (100 µg/ml) (N = 4); ●--●: poly I:C (50 µg/ml) (N = 4); △ ··· △: poly I:C (4 µg/ml) (N = 4); ▲-·-▲: poly I:C (0.8 µg/ml) (N = 4); □--□: medium control (N = 4).

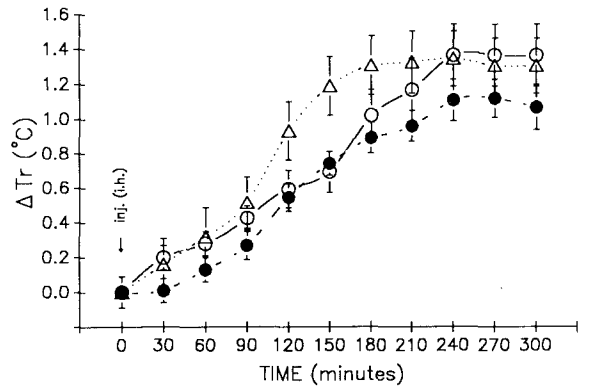

Figure 4. The mean (±SEM) change in rectal temperature of rabbits injected intrahypothalamically (inj. i.h.) with control supernatants from poly I:C-treated monocytes (3×10^6 cells/ml) alone, or experimental supernatants from monocytes (3×10^6 cells/ml) treated with poly I:C plus indomethacin or poly I:C plus cycloheximide. ○···○: Poly I:C (100 µg/ml) control (N = 4); ●--●: poly I:C (100 µg/ml) + indomethacin (50 µg/ml) (N = 4); △···△: poly I:C (100 µg/ml) + cycloheximide (50 µg/ml) (N = 4).

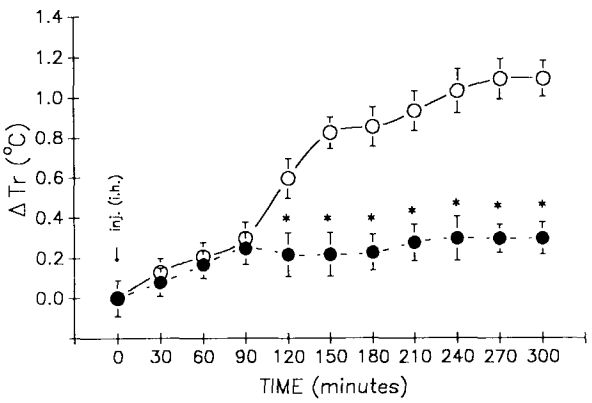

Figure 5. The mean (±SEM) change in rectal temperature of rabbits injected intrahypothalamically (inj. i.h.) with control supernatants obtained from poly I:C-treated monocytes incubated at 37 °C, or experimental supernatants obtained from poly I:C-treated monocytes incubated at 70 °C for 30 min. *Significantly different from corresponding control values (poly I:C control), $p < 0.05$ (Student's t-test). ○···○: Poly I:C (100 µg/ml) control; ●- -●: poly I:C (100 µg/ml), 70 °C, 30 min.

PGE_2, the supernatants from the poly I:C-treated monocytes were assayed for the presence of these substances. It was found that no interferon activity was detectable in the supernatants of either poly I:C-treated or medium-treated monocytes (table). The levels of interleukins, tumor necrosis factor of PGE_2 obtained from the supernatants of poly I:C-treated monocytes were not different from those obtained using medium-treated human blood monocytes.

There is evidence that fever can be caused by the action of interleukin-1[15]. However, interleukin-1 is not the only leukocyte product that induces fever; tumor necrosis factor (cachectin) and interferon also induce fever in humans and animals. The present results showed that the pyrogenic responses to supernatants from the poly I:C-stimulated human blood monocytes was characteristic of endogenous pyrogen in that it was brief and monophasic and was destroyed by heating the supernatants at 70 °C for 30 min. However, the pyrogen production was unimpaired when the incubations were carried out in the presence of cycloheximide or indomethacin. Furthermore, incubation of human blood monocytes with poly I:C did not stimulate the production of either interferon, interleukins, tumor necrosis factor or PGE_2 in the supernatants. These observations indicate that poly I:C stimulates the endogenous release of certain *unknown* pyrogens from human blood monocytes in vitro.

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Effect of poly I:C on PGE_2 and cytokine production by human peripheral blood mononuclear cells[a]

Treatment	IFN (IU/ml)	IL-1-α (pg/ml)	IL-1-β (pg/ml)	IL-6 (pg/ml)	TNF (pg/ml)	PGE_2 (ng/ml)
Medial control	<5	1051.0 ± 88.3 (N = 7)	30.6 ± 4.7 (N = 6)	273.1 ± 26.8 (N = 6)	33.8 ± 5.0 (N = 6)	2.42 ± 0.08
Poly I:C (100 µg/ml)	<5	1174.0 ± 106.0 (N = 7)	21.6 ± 5.5 (N = 5)	372.9 ± 45.3 (N = 6)	41.3 ± 1.0 (N = 6)	1.53 ± 0.03

[a]Human PBMC (3×10^6/ml) were incubated with 100 µg/ml of poly 1:C at 37 °C for 1 h, and then the cell suspensions were washed three times with DMEM as described under 'Materials and methods'. The values represent the mean ±SE of three separate experiments.