Tropical Medicine, 26 (1), 37–41, March, 1984

Studies on Pyrogenic Fever Induced by Granulocytes-Free Leukocytes in Rabbits

Mariko FUJIWARA, Jun IWAMOTO, Nobu OHWATARI, Katsuhiko TSUCHIYA and Mitsuo KOSAKA

Department of Environmental Physiology, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, Japan

Tadamichi YANAGI and Yoshiro TSUJI

Department of Pediatrics, School of Medicine, Nagasaki University, Nagasaki 852, Japan

Soeliadi HW*

Department of Internal Medicine, Medical Faculty of Gadjah Mada University, Indonesia

Abstract: The ability of rabbits' mononuclear cells to release a leukocytic pyrogen (LP) *in vitro* and its pyrogenecity were studied in the rabbits. Crude LP was obtained from granulocytes-free leukocytes as follows; the mixed cells of lymphocytes and monocytes had been separated on Ficoll-Conray gradients from whole blood of rabbits, and these cells were sensitized with bacterial lipopolysaccharide (LPS) in RPMI 1640 solution for two hours at 37°C in the presence or absence of acetylsalicylate. To estimate the pyrogenecity of LP, these crude pyrogens were intravenously applied to conscious rabbits. A typical response to LP was characterized by a rapid onset and short lasting of fever; after the short delay of 10 to 15 minutes, biphasic fever curve was developed following the two phases of decreased ear skin temperature. When acetylsalicylate, an inhibitor of cyclooxygenase, was added to culture medium before beginning of incubation, the first slight rise of rectal temperature became smaller or disappeared, and the duration of initial fall of ear skin temperature was shortened. Further, underlying mechanism of these crude LP-induced fever was discussed in this paper.

Key words: mononuclear cells, leukocytic pyrogen (LP), fever pattern, interleukin 1 (IL 1)

(1) Febrile phenomena have been extensively studied by investigators both in the physiological field and in the immunological field (Mendelsohn, 1964; Atkins,

Received for publication, March 21, 1984.
Contribution No. 1388 from the Institute for Tropical Medicine, Nagasaki University.
*Participant in the Group Training Course of Research in Tropical Medicine sponsored by Japan International Cooperation Agency (JICA).

1982, 1983). The physiologists were interested in the thermoregulatory mechanism during fever (Cooper *et al.*, 1964; Cabanac and Massonnet, 1974), and the immunological investigators paid attentions to the defence reaction against the infectious diseases (Epstein *et al.*, 1951). Since there had been many evidences that fever was caused by many kinds of polypeptides released by leukocytes (Fessler *et al.*, 1961; Bodel and Miller, 1978), these fever-inducing agents were usually called as leukocytic pyrogen (LP). In 1953, LP was first obtained from sterile suspensions of rabbits peritoneal exudate cells by injecting sodium chloride solution (Bennett and Beeson, 1953). Because these cells usually obtained more than 95% granulocytes, most works about the release and characterization of LP have been carried out with granulocytes (Hahn *et al.*, 1970; Ogawa and Kanoh, 1975). However, by remarkable progresses in immunological studies, it was shown that interleukin 1 (IL 1), peptide of monocytes origin that formerly called as lymphocytes activating factor, was responsible for fever development. There are some substantial evidences, based on functional and biochemical properties, to indicate that LP and IL 1 are identical (Rosenwasser *et al.*, 1979; Murphy *et al.*, 1980 b). Therefore, in the present study the pyrogenecity of LP derived from granulocytes-free leukocytes, monocytes and lymphocytes, was tested in conscious albino rabbits.

(2) Crude LP was produced *in vitro* as follows: lymphocytes and monocytes were separated from whole blood on Ficoll-Conray gradients, and these cells in one layer were aspirated and washed several times in phosphate buffer solution (PBS: Nissui Seiyaku Co.) of pH 7.4 and suspended on RPMI 1640 culture solution (Nissui Seiyaku Co.) at about 4×10^6 cells/ml. In a 5% CO_2 incubator, these cells were stimulated with 5ng/ml LPS pyrogen from *E. coli* (No. 0111:B8, SIGMA) for two hours at 37°C. After incubation, culture fluid was filtered through a 0.45μm millipore filter (Millipore Co., Besford, MA) to remove any cells, and the undiluted supernatant containing the crude pyrogen was used as LP. For the biological assay, unanesthetized albino rabbits weighing between 2.0kg and 2.5kg were used. The animals were lightly restrained in the prone position, and rectal and ear skin temperatures were continuously recorded with the thermistor probes. The respiratory rate was detected from the resistance changes of strain gauge. Oxygen consumption was measured by using a Benedict-Roth respirometer (13.5 liters, bell factor: 41.1ml/min). All experiments were carried out in a climatic chamber of which air temperature and relative humidity were kept constant at 28°C, 60%, respectively. Crude LP of 3ml/kg was intraveneously injected through the retroauricular vein of the rabbit.

(3) Febrile reaction was recorded after administration of crude LP. Typical pyrogenic responses are shown in Fig. 1. Rectal temperature (Tr) began to rise within 10 minutes after the intravenous injection of 3ml/kg crude LP. After the start of febrile reaction, two falling phases of ear skin temperature (Te) were observed. An initial

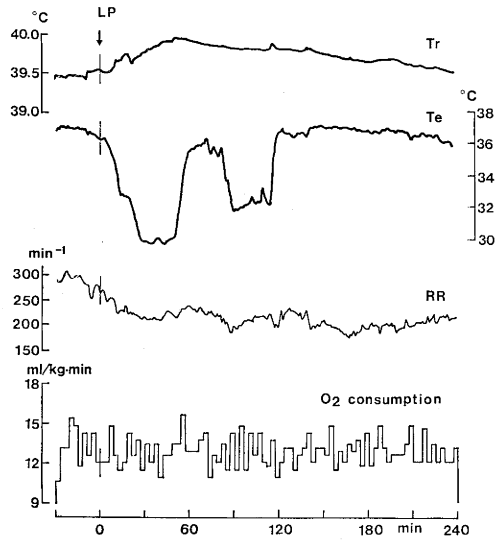

Fig. 1. Febrile responses of a rabbit to intravenous injection of crude LP at an arrow. Changes in rectal temperature (Tr), ear skin temperature (Te), respiratory rate (RR) and oxygen consumption (O_2 consumption) are shown.

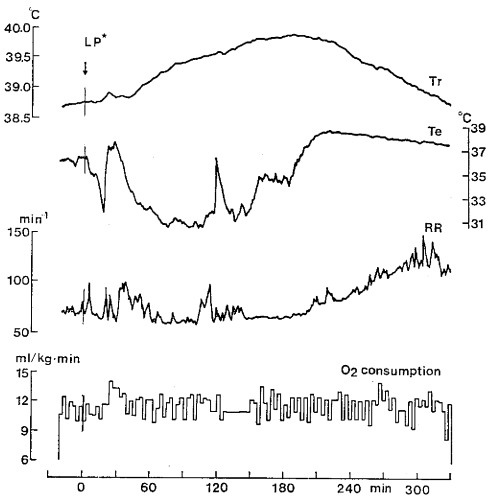

Fig. 2. Febrile responses of a rabbit to intravenous injection of crude LP which was treated with 50μg/ml aspyrine (LP*). Changes in rectal temperature (Tr), ear skin temperature (Te), respiratory rate (RR), and oxygen consumption (O_2 comsumption) are shown.

falling phase of Te preceded to the slight rise of Tr was rapid, but this peripheral vasoconstriction usually recovered within 30 minutes. After the short latency, the second drop of Te occured after subsequent elevation of Tr. The duration of LP induced fever observed in the present experiment was about two hours. However, no significant changes were observed both in respiratory rate (RR) and in oxygen consumption (O_2 consumption). It is still doubtful that this fever might be due to prostaglandin produced during incubation or not. To clarify this question, 50μg/ml acetylsalicylate (aspyrine), an inhibitor of cyclooxygenase, was added to the culture medium of monocytes and lymphocytes before beginning of incubation. Fig. 2 shows the febrile patterns of aspyrine-treated LP. Initial rise of Tr became smaller or disappeared, and duration of the first fall of Te was shortened compared with those of fever induced by aspyrine-free LP. From these facts, it was assumed that the initial falling phase of Te elicited from extrinsic prostaglandin produced during incubation, and the second falling phase of Te might be due to fever induced by crude LP.

(4) It is generally considered that exogenous pyrogen acts on the reticulo-endothelial system to produce endogenous pyrogen, which affects on the central thermosensitive neurones or somewhere in the thermoregulatory system (Atkins, 1960), and this liberated endogenous pyrogen is thought to be an essential mediator of fever (Milton, 1976). In

addition, this endogenous pyrogen has been reported to be a product of granulocytes. However, it has shown recently that the granulocytes, though they functioned normally in other ways, did not generate detectable amounts of endogenous pyrogen *in vitro* (Hanson *et al.*, 1980; Atkins, 1983). Using phagocytosis of *stapylococci* as a stimulus, Murphy and his associates (1980a) showed that production of endogenous pyrogen in mixed populations of leukocytes can be entirely attributed to their monocytes contents. Furthermore, evidences are accumulating that LP derived from monocytes was identical to interleukin 1 (IL 1), which has been recognized as an activator of helper T-cells (Duff and Durum, 1983). Because of the difficulties of separating sufficient amounts of monocytes from mixed cell populations, both monocytes and lymphocytes were cultured in the present experiments. So that, clude LP intermingled IL 1, peptides of monocytes origin, and IL 2, the product of T-lymphocytes. It must be necessary to selective isolation of monocytes, and to examine its properties such as T-cell prolifaration are completely identical to IL 1 or not. Further experiments must be introduced to clarify the thermoregulatory mechanism during fever by means of the purified IL 1.

REFERENCES

1) Atkins, E. (1960): Pathogenesis of fever. Physiol. Rev. 40, 580–646.

2) Atkins, E. (1982): Fever: Its history, cause, and function. Yale J. Biol. Med., 55, 283–285.

3) Atkins, E. (1983): Fever — New perspectives on an old phenomenon. N. Engl. J. Med., 308, 958–960.

4) Bennet, I. L. & Beeson, P. B. (1953): Studies on the pathogenesis of fever. I. The effect of the injection of extracts and suspensions of uninfected rabbit tissues upon the body temperature of normal rabbits. J. Exp. Med., 98, 477–492.

5) Bodel, P. & Miller, H. (1978): A new sensitive method for detecting human endogenous (leukocyte) pyrogen. Inflammation, 3, 103–110.

6) Cabanac, M. & Massonnet, B. (1974): Temperature regulation during fever: Change of set point or change of gain? A tentative answer from a behavioural study in man. J. Physiol., 238, 561–568.

7) Cooper, K. E., Cranston, W. I. & Snell, E. S. (1964): Temperature regulation during fever in man. Clin. Sci., 27, 345–356.

8) Duff, G. W. & Durum, S. K. (1983): The pyrogenic and mitogenic actions of interleukin-1 are related. Nature, 304, 449–451.

9) Epstein, H. C., Hochwald, A. & Ashe, R. (1951): Salmonella infections of the newborn infant. J. Pediatr., 38, 723–731.

10) Fessler, J. H., Cooper, K. E., Cranston, W. I. & Vollum, R. L. (1961): Observations on the production of pyrogenic substances by rabbit and human leukocytes. J. Exp. Med., 113, 1127–1140.

11) Hahn, H. H., Cheuk, S. F., Elfenbein, C. D. D. & Wood, W. B.Jr. (1970): Studies on the pathogenesis of fever. XIX. Localization of pyrogen in granulocytes. J. Exp. Med., 131, 701–710.

12) Hanson, D. F., Murphy, P. A. & Windle, B. E. (1980): Failure of rabbit neutrophils to secrete endogenous pyrogen when stimulated with staphylococci. J. Exp. Med., 151, 1360−1370.
13) Mendelsohn, E. (1964): Heat and life: In: The development of the theory of animal heat. Harvard University Press, Cambridge.
14) Milton, A. S. (1976): Prostglandins in fever. In: Brain dysfunction in infantile febrile convulsions. (eds) Brasier, M. A. B. & Coceani, F., Raven Press, New York.
15) Murphy, P. A., Hanson, D. F., Simon, P. L., Willoughby, W. F. & Windle, B. E. (1980a): Properties of two distinct endogenous pyrogens secreted by rabbit macrophages. In: Microbiology. (ed) Schlesinger, D.
16) Murphy, P. A., Simon, P. L. & Willoughby, W. F. (1980b): Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte activating factors made by rabbit alveolar macrophages. J. Immunol., 124, 2498−2501.
17) Ogawa, Y. & Kanoh, S. (1975): Machanisms of release of leucocytic pyrogen. Folia pharmacol. japon., 71, 263−271. (*In Japanese*)
18) Rosenwasser, L. J., Dinarello, C. A. & Rosenthal, A. S. (1979): Adherent cell function in murine T-lymphocyte antigen recognition. IV. Enhancement of murine T-cell antigen recognition by human leukocytic pyrogen. J. Exp. Med., 150, 709−714.

単球及びリンパ球由来白血球性発熱物質によるウサギ発熱に関する研究

藤原真理子，岩元　純，大渡　伸，土屋勝彦，小坂光男（長崎大学熱帯医学研究所，環境生理）
柳　忠道，辻　芳郎（長崎大学医学部小児科学教室）
Soeliadi Hw（ガジャマダ大学医学部内科学教室）

顆粒球を含まない白血球から生成した発熱物質を用いてウサギの発熱曲線を解析した．ウサギ全血から Ficoll-Conray 比重遠心法にて単球とリンパ球を分離し，37°C の炭酸ガス培養器中で，大腸菌由来の lipopolysaccharide (LPS) を 2 時間感作させて，内因性発熱物質 (LP) を含む培養液を得た．この培養上清液をウサギに静注したところ，10〜15分の短い潜時を経て二峰性の発熱が現われた．この直腸温上昇に伴い，耳介皮膚温の二度にわたる下降が観察された．発熱の持続時間は通常 2 〜 3 時間以内であった．しかしながら，シクロオキシゲナーゼの合成阻害剤であるアセチルサリチル酸を培養液に添加して得られた LP 投与では，一峰目の直腸温のわずかな上昇は，減少あるいは消失し，それに伴い，耳介皮膚温の初期下降相の持続時間も短縮された．以上の結果をもとに，内因性発熱物質の作用について新たな考察を加えた．

熱帯医学　第26巻　第1号，37−41頁，1984年3月