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SHORT COMMUNICATION

Interferon-beta, but not tumor necrosis factor-alpha, production in response to poly I:C is maintained despite exhaustive exercise in mice

Masataka Uchida · Eri Oyanagi · Michael J. Kremenik · Junzo Sasaki · Hiromi Yano

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Abstract It remains unclear whether immune response to viral infection is inhibited by severe exercise. We determined whether exhaustive exercise inhibits interferon (IFN)-β and tumor necrosis factor (TNF)-α production after injection of synthetic double-stranded (ds) RNAs, a polyriboinosinic polyribocytidylic acid (poly I:C), as viral infection model. Male C3H/HeN mice, which were divided into exhaustive-exercised and non-exercised groups, were injected with poly I:C (5 mg/kg). Although TNF-α in response to poly I:C was significantly inhibited by exhaustive exercise, IFN-β was no different in both groups. In in-vitro experiments, catecholamines inhibited poly I:C-induced TNF-α, but not IFN-β, production in macrophages. These results suggest that anti-virus cytokine IFN-β in response to poly I:C might be maintained despite severe stressful exercise.

Keywords Polyriboinosinic polyribocytidylic acid · IFN-β · TNF-α · Toll-like receptor 3 · C3H/HeN mice

Introduction

It is believed by open-window theory that intense exercise induces immune suppression [1]. In fact, we and other researchers have found that high concentrations of pro-inflammatory cytokines, for example tumor necrosis factor (TNF)-α, in plasma were strongly inhibited by exhaustive exercise before challenge of rodents with lipopolysaccharide (LPS), a gram-negative bacteria component [2–4]. In addition, it has been reported that exercise stress might be associated with increased susceptibility to viral infections [5, 6]. However, changes in pathogen-induced type I interferons (IFN-α/β), which are very important in anti-virus response, after severe exercise are little known, although we reported that exhaustive exercise induced depression of IFN-α against injection of imidazoquinoline resiquimod (R-848), a ligand of the toll-like receptor (TLR)7 which responds to single-stranded (ss) RNA viruses to trigger IFN production in mice [7].

Polyriboinosinic polyribocytidylic acid (poly I:C) is a potent inducer of IFN-β [8]. Poly I:C-induced immune activation is very similar to virus double-stranded (ds) RNA-induced immune activation, because both poly I:C and viral dsRNA bind to TLR3 and then activate IFN-β [9]. The dsRNA can be generated during viral infection as a replication intermediate for ssRNA viruses or as a by-product of symmetrical transcription in DNA viruses [10]. In fact, TLR3 protects against infection by ssRNA viruses [11, 12]. Thus, the role of TLR3 in antiviral response after exhaustive exercise might be important, and it is possible that changes in immune function after viral infection with exercise can be observed to study poly I:C injection in exercised or non-exercised animals.

The purpose of this study was to determine whether exhaustive exercise attenuates the increase in plasma concentration of IFN-β and TNF-α after poly I:C injection in mice. We hypothesized that exhaustive exercise could attenuate the increase in IFN-β and TNF-α in response to poly I:C.

M. Uchida · E. Oyanagi · J. Sasaki
Department of Cytology and Histology, Okayama University Graduate School, Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikatacho, Okayama 700-8558, Japan

M. J. Kremenik · H. Yano (✉)
Department of Health and Sports Science, Kawasaki University of Medical Welfare, 288 Matsushima, Kurashiki, Okayama 701-0193, Japan
e-mail: yanohiro@mw.kawasaki-m.ac.jp

Materials and methods

Ten-week-old male C3H/HeN mice ($n = 23$) were housed individually in cages and maintained on a 12:12 h light:dark cycle with free access to food and water. The experimental procedures followed the guidelines set forth in the Care and Use of Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan, and the experimental procedures and housing conditions were approved by the DHSS (Animal Care and Use Committee) of Kawasaki University of Medical Welfare (#HSS070006). The mice were randomly divided into two groups. One group contained exhaustive exercising mice (EX, $n = 12$), and the other group contained non-exercising mice (N-EX, $n = 11$). The EX mice were run on a treadmill to exhaustion, and N-EX mice were kept at rest [4, 7]. Mean time to exhaustion for EX mice was 65 ± 2 min. Each group was injected with poly I:C (5 mg/kg) [13] immediately after the exhaustive exercise or rest. Each mouse was lightly anesthetized with inhalant isoflurane before i.v. injection via the orbital eye vessel. Blood samples were collected from each eye vessel pre-exercise and 1, 3, and 6 h after poly I:C injection in mice.

RAW264 cells, a mouse-derived macrophage cell line, were obtained from the Cell Bank Riken Bioresource Center (Ibaraki, Japan). These cells were cultured in DMEM containing 10% FCS supplemented with 200 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2. The RAW 264 cells (2×10^4/well) in 96-well plates were pre incubated for 24 h and then stimulated for 30 min with phosphate-buffered saline (PBS) containing DMSO, as vehicle, epinephrine (1 μM), norepinephrine (1 μM), and dopamine (1 μM). They were then challenged with poly I:C (10 μg/ml) for 6 h.

The plasma and supernatants were collected and then stored at −40°C until analysis of TNF-α, IFN-α, and IFN-β using mouse ELISA kits.

Results and discussion

The plasma TNF-α concentration in N-EX mice was greatly increased 1 and 3 h after poly I:C injection ($p < 0.01$ and $p < 0.05$, respectively, Fig. 1a). In EX mice, however, elevation of the TNF-α concentration in plasma was not as high as that in the N-EX group 1 and 3 h after poly I:C injection ($p < 0.01$ for both). This phenomenon, that the poly I:C-induced increase in plasma TNF-α concentration is attenuated by prior exhaustive exercise, seems to be very similar to that of LPS-induced TNF-α response in plasma [2–4]. Therefore, intense exercise might inhibit poly I:C-induced pro-inflammatory cytokine production, and might then increase the risk of infection with viruses.

In fact, however, activation of the type I IFNs is very important in anti-virus response [9]. We also focused on poly I:C-induced type I IFNs, i.e. on IFN-α and β production. Interestingly, plasma IFN-β concentration in EX mice obviously increased 3 h after poly I:C challenge, as did that in N-EX mice ($p < 0.01$ for both; Fig. 1b), although plasma IFN-α in both EX and N-EX mice, was not detected in this experimental model (data not shown). This is, to our knowledge, the first study to demonstrate that IFN-β production in response to poly I:C is maintained, even though the mice are subjected to exhaustive exercise stress. A previous study showed that exhaustive exercise inhibited INF-α production in mice when the mice were treated by R-848 as activator of TLR7 [7]. A difference function between poly I:C/TLR3 and R-848/TLR7 might have occurred via a

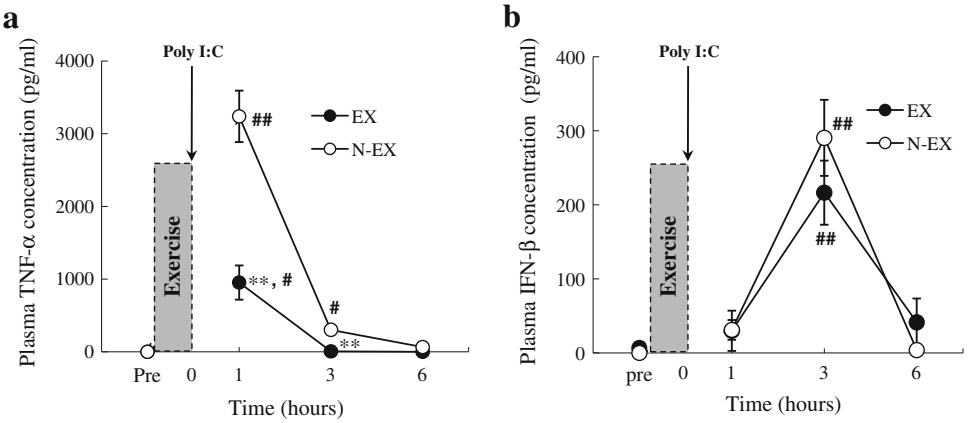

Fig. 1 Changes in TNF-α (**a**) and IFN-β (**b**) concentration in plasma before and after poly I:C injection in exhaustive exercised (EX: *filled circles*, $n = 12$) and non-exercised (N-EX: *open circles*, $n = 11$) mice. Data are expressed as means ± SEM. The data were analyzed by two-way repeated-measures analysis of variance (ANOVA) and post-hoc Bonferroni's tests were performed. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. pre-injection of poly I:C in each group, and $^{**}p < 0.01$ vs. N-EX at each point

myeloid differentiation factor (MyD)88-independent or dependent pathway. Moreover, TLR3 protects against infection by ssRNA viruses [11, 12] despite viral ssRNA binding to TLR7 [10]. One possibility is that TLR3 signaling is not attenuated, despite strenuous exercise as the most important second defense system, because the dsRNA can be generated during viral infection as a replication intermediate for ssRNA viruses or as a by-product of symmetrical transcription in DNA viruses [10]. These results suggest that exhaustive exercise may do little to affect viral infection-induced anti-viral cytokine production.

However, it remains unclear why exhaustive exercise inhibits TNF-α, but not IFN-β, in response to poly I:C. It is well known that exercise induces production of catecholamine and other hormones. Furthermore, Kitamura et al. [3] suggested that exercise-induced catecholamines are responsible for exercise-induced suppression of pro-inflammatory cytokine production. Thus, one possibility is that intense exercise-induced pro-inflammatory cytokine suppression is regulated by catecholamines. Therefore, in the next experiment, to clarify whether catecholamine regulated viral infection-induced anti-viral cytokine production, we studied the effect of epinephrine, norepinephrine, and dopamine on poly I:C induced IFN-β production in vitro. Poly I:C-induced TNF-α production by RAW264 cells was significantly inhibited by treatment with epinephrine ($p < 0.05$) and dopamine ($p < 0.01$) (Fig. 2a, c). It has been reported that β-adrenergic agonists suppress, and α-adrenergic agonists augment, pathogen-stimulated TNF-α production and its gene expression [14]. Norepinephrine has high affinity for both α and β-adrenergic receptors, whereas epinephrine has high affinity for β-adrenergic receptors only [15]. Therefore, β-adrenergic receptors, which have affinity for both epinephrine and norepinephrine, may modulate the effects of catecholamines on the exercise-induced changes in TNF-α in response to poly I:C. Our results with dopamine also accorded well with recent studies which discovered that dopamine suppresses production of pro-inflammatory cytokines (IL-12 and TNF-α) via the β-adrenergic receptor, but not the dopamine receptor [16, 17].

However, we did not observe a significant difference between IFN-β production in cells treated with several catecholamines and vehicle. Although high-dose treatment (10–100 μM) with catecholamines inhibits IFN-β synthesis and/or its mRNA expression after LPS or CpG DNA stimulation [18, 19], in our experimental model we did not observe inhibition of poly I:C-induced INF-β production by catecholamine treatment (Fig. 2d–f). In addition, the doses of epinephrine, norepinephrine, and dopamine (1 μM) might be similar to exercise-induced plasma catecholamine levels

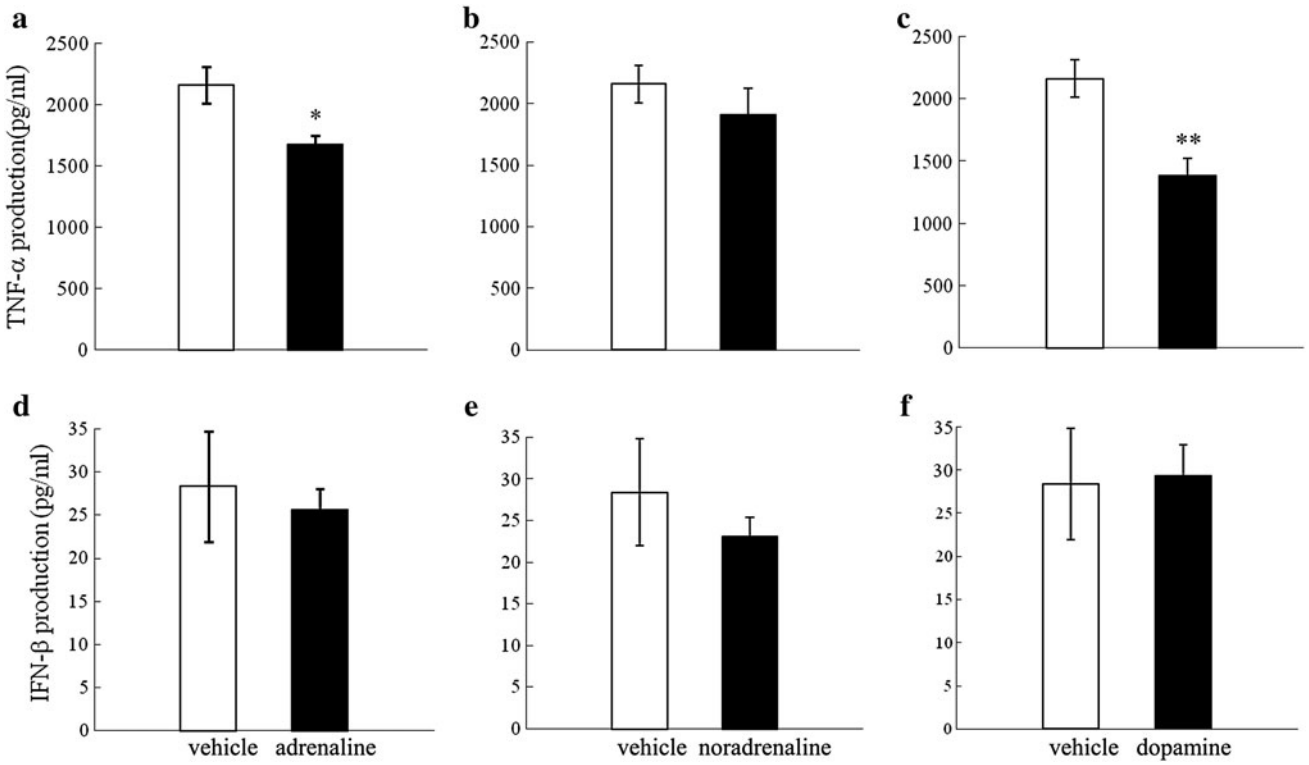

Fig. 2 Effects of catecholamine on poly I:C-induced TNF-α (**a**–**c**) and IFN-β (**d**–**f**) production in RAW 264 cells. RAW264 cells ($n = 8$–11) were treated with epinephrine (1 μM; **a**, **d**), norepinephrine (1 μM; **b**, **e**), dopamine (1 μM; **c**, **f**), or vehicle 30 min before poly I:C (10 μg/ml) stimulation for 6 h. Results are expressed as the means ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. vehicle. Statistical analysis was performed with the Student *t* test

[3, 20]. There was common evidence that poly I:C-induced INF-β production is maintained despite intense exercise in vivo and the phenomenon is reproduced in an in-vitro experiment, which is a test of catecholamine treatment of poly I:C stimulated macrophages. Indeed, it has been reported that exercise did not inhibit both IFN-β mRNA expression and its protein on infection with herpes simplex virus type I or influenza virus [21, 22].

It remains to be determined which specific cell type is responsible for the major source of IFN-β in response to poly I:C, because TLR3 is expressed in a variety of cells [10], and IFN-β is also secreted by many cell types, including macrophages, fibroblasts, epithelial cells, dendritic cells, and others [23, 24]. Moreover, poly I:C-induced IFN-β production by retinoic acid-inducible gene (RIG)-I and/or melanoma differentiation-associated gene (MDA) 5, in addition to TLR3 [10], during and after exercise, are quite intriguing issues that remain to be clarified.

In summary, this study set out to determine whether exhaustive exercise attenuates the increase in plasma concentrations of IFN-β and TNF-α after poly I:C injection in mice. Although TNF-α concentration in exercised mice was significantly lower than that in non-exercised mice, the poly I:C induced increase in IFN-β concentration was not significantly different in both groups. Furthermore, in in-vitro experiments, previous treatment with both adrenaline and dopamine inhibited poly I:C-induced TNF-α production but not IFN-β production in macrophages. Taken together, these results suggest that anti-virus cytokine IFN-β production in response to pathogen stimulation might not be affected by severe stressful exercise.

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