EOSINOPHILIA IN PHYSICAL EXERCISE STRESSOR: PATHOBIOLOGY OR PHYSIOBIOLOGY?

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ABSTRACT

Eosinophilia plays an important role in asthmatic airway inflammation or allergy. Exercise dose that can increase eosinophil in blood remains controversial. Blood eosinophil measurement was carried out in males of 17 - 22 years old who performed several intensive physical exercises. They were divided into the following groups: Group I, physical exercise program group, comprising high school students who involved in a physical exercise study on the effect of 80-90% maximum heart rate on immunity; Group II, the athletes of Pekan Olahraga Nasional *XVI 2004 in East Java; Group III, football athletes prepared for National Football League, and control data were taken from students who did not perform intensive exercise. The results of blood eosinophil measurement were as follows: 0.3158 ± 0.3238; 0.3475 ± 0.2285; 0.4305 ± 0.3146; 0.2760 ± 0.1873 (x10e3 /uL). More strenuous physical exercise was found to increase blood eosinophil, although not significant (ANOVA, p > 0.05). The eosinophilia itself may either aggravate allergic disease or even as a reflection of immunity status improvement, a possibility that worth to be investigated further.*

Keywords: *eosinophilia, physical exercise program*

INTRODUCTION

Eosinophil is a leukocyte which contains eosinophilic granules strongly stained with eosin. The granules in eosinophil contain cationic proteins which strongly bind to eosin. The morphological characteristic of eosinophil is the existence of crystalloid granules. Eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and major basic protein (MBP) are known to be major protein-mediators derived from activated eosinophils. ECP and EDN are found in matrix of granules in eosinophils whereas MBP is found in core of granules. ECP and EDN are members of the ribonuclease A superfamily. ECP and MBP have high cytotoxicity. These three proteins are highly cationic proteins with pH 10.8-10.9 (Plager DA, 1999). Activated eosinophils play an important role in the late asthmatic response and in the asthmatic airway inflammation. As ECP is secreted from activated eosinophils, ECP can be a marker of eosinophil activation and degranulation (Plager DA, 1999; Boix E, 2001). ECP is a basic secretion protein involved in the immune response system. ECP levels in biological fluids reflect ongoing eosinophilic airway inflammation and are used for the clinical monitoring and diagnosis of asthma. Eosinophil in blood may experience turnover. The migration of eosinophils from the bone marrow to the blood takes about 3-4 days (Giembycz and Lindsay, 1999). Recently, the increase of eosinophil count in blood is mostly assumed as the presence of allergic reaction. However, the significance of increased blood eosinophil count in physical exercise is still less understood. Facts showed that various mechanisms are closely related with the emergence of allergic reaction. Physical exercise may enhance resistance to upper respiratory tract infection (Pedersen BK, 2000), and even elite athletes also showed an increase of IgG and IgM (Verde TJ, 1992), and the reduction of allergic recurrence (Pedersen BK, 2000). Correlation between reduced allergic recurrence and reduced eosinophil count in blood has also not been reported. Based on above analysis, physical exercise stressor may result in the reduction of eosinophil count in blood. A study showed that physical exercise stressor could result in increased eosinophil count, which had adverse effect on the body. The increased eosinophil would migrate and increasing accumulation in tissue could even increase allergic reaction (Susan L, 2005).

MATERIAL AND METHODS

This study was an observational research to individuals who carried out physical exercise program. Group I consisted of high school students who received physical exercise 80-90% of maximum heart rate. Group II consisted of *silat* athletes who participated in *Pekan Olahraga Nasional* XVI in 2004, East Java, while football athletes prepared for competition in National Football League were recruited in Group III. Control was taken from high school students who did not perform intensive physical exercise program. The program in each group was conducted for 10 weeks.

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Respondents in each group comprised 20 individuals, taken randomly from the population of each group.

Health status of all observed groups was examined in laboratory. Erythrocyte count, hemoglobin level, leukocyte count, hepatic function (SGOT and SGPT), and renal function (creatinine serum) served as moderator variables. The main variable in this study was blood eosinophil, which was taken from cubitous veins 24 hours post-exercise at the end of week 10. Eosinophil measurement was done using Technicon-3 peroxidase method.

RESULTS

The results of moderator variables measurement in groups I, II, III and control were as follows: (1) erythrocyte count (6.05 ± 0.23; 5.55 ± 0.33; 5.50 ± 0.32; 56.05 ± 0.13 x10.e6/uL, $p > 0.05$), (2) leukocytes (6.62 ± 1.54; 6.52 ± 0.56; 6.42 ± 1.04; 6.70 ± 0.90 x 10e3/uL, $p > 0.05$), (3) hemoglobin (14.51 ± 0.74; 14.05 ± 0.70; 13.96 ± 1.24; 14.25 ± 1.44 g/dL, $p > 0.05$), (4) hepatic function SGOT (12.50 ± 1.14; 13.50 ± 1.54 ; 13.96 ± 0.95 ; 13.68 ± 1.15 , $p > 0.05$), hepatic function SGPT (9.50 ± 1.24; 10.15 ± 0.94; 13.20 ± 1.44; 12.53 ± 2.22 U/L $p > 0.05$) and (5) creatinine serum (0.86 ± 0.14; 0.96 ± 0.34; 1.06 ± 0.54; 0.90 ± 0.24 mg%, $p > 0.05$). The moderator variables were found not to have difference between groups and normal. The mean of eosinophil count in three groups and control can be seen in the following table.

Table 1. The description of eosinophil count

Groups	N	Mean	Standard Deviation
Control	20	.2760	.1873
Treatment 1	22	.3158	.3238
Treatment 2	20	.3475	.2285
Treatment 3	20	.4305	.3146
Total	82	.3418	.2722

Note:
Eosinophil unit = x 10 e3/uL. ANOVA (p = 0.325)

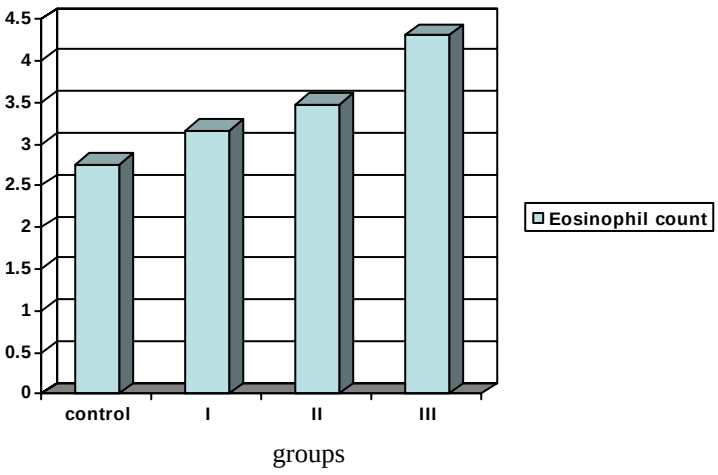

Figure 1. The mean of eosinophil count in three groups and control

DISCUSSION

The results showed that the eosinophil count was not different ($p > 0.05$). However, the strenuous physical exercise brought about an increase above normal level. Several studies showed that moderate endurance exercise could increase total leukocytes, granulocyte, monocyte, lymphocyte, natural killer cell, cell proliferation in response to mitogens and serum immunoglobulin levels (Shephard RJ and Shek PN, 1994). Another study proved that aerobic exercise did not result in chemotaxis and bactericidal activity increase, which is played by eosinophil, while anion superoxide is mostly secreted in strenuous exercise, both in trained and athlete groups (Wolach B, 2000). The increase of blood eosinophil count was found in strenuous exercise. Previously, Hack V (1994) had proved that vigorous exercise increased polymorphonuclear (PMN) cell count. Nevertheless, in such exercise the immune cell function is poor, natural killer cell cytotoxicity (NKCC) is low, lymphocyte response to mitogens in culture is also poor, and there is frequent immunoglobulin deficiencies, most often IgG1 and IgG3(Hack V, 1994; Chouker A, 2005).

Physical exercise may result in the increase of various cytokines in blood. It has been established that proinflammatory cytokines may also increase in blood either in infectious or non-infectious condition (Farina L and Winkelman C, 2005). Physical exercise may also increase gene expression (IL-1 and IL-5) in peripheral blood mononucleus cells (Connolly PH, et al, 2004; Zieker D, et al., 2005), and revealed upregulated growth and repair genes (Connolly PH, et al, 2004). Cytokines can serve as immunomodulator or immunocompetent cells autocrin. Moreover, both signaling types may also act not only as stimulator, but also as negative feedback mechanism (Frost and Lang, 2004).

Cell viability, multiplication, and differentiation to the various hematopoietic cell lineages are induced by a multigene cytokine family, and hematopoiesis is controlled by a network of interactions between these cytokines (Sachs L and Lotem, 1994). This network includes positive regulators such as colony-stimulating factors and interleukins, and negative regulators such as transforming growth factor-beta and tumor necrosis factor (Sachs L and Lotem J, 1994). IL-5 activated the Jak 2-STAT 1 signaling pathway in eosinophils (Pazdrak, 1995). IL-5 plays a pivotal role in growth and differentiation of eosinophils. The signal transduction mechanism of IL-5R is largely unknown (Stafford S, 2002). IL-5 specifically facilitated the terminal differentiation and proliferation of eosinophils. The synergistic effects of IL-5 and colony-stimulating factors on the expansion of eosinophils are supposed to contribute to the urgent mobilization of eosinophils at the time of helminthic infections and allergic responses (Yamaguchi Y, 1988). Others investigation, monoclonal anti-IL-5 reduces peripheral blood eosinophils without altering the distribution and activation status of lymphocytes (Büttner C, 2003). IL-5 was an important mediator of eosinophil. IL-5-released stimulated by IL-2 (Yamaguchi Y, 1990). In contrast, eosinophil can produce a soluble substance that enhances lymphocyte IL-5 secretion (Metwali A, 1993). IL-5 and GM-CSF may rise in asymptomatic asthmatic condition (Brown PH, 1991), although the increase of IL-5 in physical exercise does not bring about asthmatic or allergic symptoms. One observation indicated that in allergic condition, there is an increase of IL-4 and TNF-alpha (Zangrilli JG, 2002), while IL-5 was able to maintain cellular apoptotic process (Zangrilli JG, 2000). Other facts showed that eosinophil could also secrete IL-1, GM-CSF, IL-3, IL-5, IL-6, IL-8, TGF-alpha and TGF-beta. The increase of eosinophil provides support degranulation for toxic effect against parasites. Autocrinely IL-5 increase eosinophil survival and activity (Abu-Ghazaleh, 1992). Thus, the increase of eosinophil count may augment immunity capability through the secretion of Eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and major basic protein (MBP). These proteins are known to be major protein-mediators derived from activated eosinophils. ECP and EDN have high cytotoxicity and the secretion of other chemical substances for modulating immunity against other immunocompetent cells (Plager DA, 1999). Additional studies have proved that interaction of signaling transduction between eosinophil and lymphocytes also contributed to immunity enhancement (Weller, 1997).

In physical exercise there is also an increase of leukotriene B4 (LTB4) and LTC4 that contribute to exercise-induced asthma (Zangrilli JG, 2002; Hilberg T, 2005). However, facts proved that all athletes in this study had no allergic or asthmatic attack. The phenomenon of chemical mediator increase also has a pathobiological potential related to eosinophil accumulation in tissues (Susan L, 2005). After exercise, there may be extracellular deposition of eosinophil granule proteins, in addition to the deposition of immune complexes and a variety of cytokines from the infiltrating cells. This appears to be one of the key biological of urticarial lesions or allergy (Kano Y, 1998; Susan L, 2005).

CONCLUSION

The phenomenon of an increase in blood eosinophil count in physical exercise program may indicate double phenomena, aggravating allergy or increasing immunity status.

REFERENCES

Abu-Ghazaleh RI, Kita H and Gleich GJ, 1992. Eosinophil activation and function in helath and disease. *Immunol Ser* 57, pp. 137-167.

Boix E, Carreras E, Nikolovski Z, Cuchillo CM and Nogués MV, 2001. Identification and characterization of human eosinophil cationic protein by an epitope-specific antibody. *Journal of Leukocyte Biology* 69, pp. 1027-1035.

Brown PH, Crompton GK and Greening AP, 1991. Proinflammatory cytokines in acute asthma. *Lancet* 338, pp. 590-593.

Büttner C, Lun A, Splettstoesser T, Kunkel G and Renz H, 2003. Monoclonal anti-interleukin-5 treatment suppresses eosinophil but not T-cell functions. *J Eur Respir* 21, pp. 799-803.

Chouker A, Demetz F, Martignoni A, Smith L, Setzer F et al., 2005. Strenuous physical exercise inhibits granulocyte activation induced by high altitude. *J Appl Physiol* 98, pp. 640-647.

Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J et al., 2004. Effects of exercise on gene expression in human peripheral blood mononuclear cells. *J Appl Physiol* 97, pp. 1461-1469.

Farina L and Winkelman C, 2005. A review of the role of proinflammatory cytokines in labor and noninfectious preterm labor. *Biological Research for Nursing* 6(3), pp. 230-238.

Frost RA and Lang CH 2004. Alteration of somatotropic function by proinflammatory cytokines. *J Anim Sci* 82, pp. E100-E109.

Giembycz MA and Lindsay MA, 1999. Pharmacology of the eosinophil. *Pharmacological Reviews* 51 (2), pp. 216-309.

Hack V, Strobel G, Weiss M and Weiker H, 1994. PMN cell count and phagocytic activity of highly trained athletes depend on training period. *J Appl Physiol* 77, pp. 1731-1735.

Hilberg T, Deigner HP, Moller E, Claus RA, Ruryk A, Glaser D, Landre JBP, Brunkhorst FM, Reinhart K, Gabriel HHW and Russwurn S, 2005. Transcription in response to physical stress-clues to molecular mechanisms of exercise-induced asthma. *The FABES J Express* Article 18, p. 3063.

Metwali A, Elliott D, Blum AM and Weinstock JV, 1993. Granuloma eosinophils enhance IL-5 production by lymphocytes from mice infected with Schistosoma mansoni. *The Journal of Immunology*, 151 (12), pp. 7048-7056.

Plager DA, Loegering DA, Weiler DA, Checkel JL, Wagner JM, Clarke NJ, et al. 1999. A novel and highly divergent homologue of human eosinophil granule major basic protein. *J Biol Chem*, 274 (20), pp. 14464-14473.

Pedersen BK and Toft AD, 2000. Effect of exercise on lymphocytes and cytokines. *Br J Sports Med*. 34, pp.:246-251.

Pazdrak K, Stafford S and Alam R, 1995. The activation of the Jak-STAT 1 signaling pathway by IL-5 in eosinophils. *The Journal of Immunology* 155 (1), pp. 397-402,

Sachs L and Lotem J, 1994. The network of hematopoietic cytokines. *Proceedings of the Society for Experimental Biology and Medicine* 206, pp. 170-175.

Shephard RJ and Shek PN, 1994. Potential impact of physical activity and sport on the immune system, a brief review. *British Journal of Sport Medicine*, 28 (4), pp. 247-255.

Stafford S, Lowell C, Sur S and Alam R, 2002. Lyn tyrosine kinase is important for IL-5-stimulated eosinophil differentiation. *The Journal of Immunology* 168, pp. 1978-1983.

Susan L, Cuvelie SL, Paul S, Shariat N, Colarusso P and Patel KD, 2005. Eosinophil adhesion under flow conditions activates mechanosensitive signaling pathways in human endothelial cells. *JEM* 202 (6), pp. 865-876

Verde TJ, Thomas SG, Moore RW, Shek P and Shepahard RJ, 1992. Immune responses and increased training of the elite athlete. *J App Physiol* 73, pp. 1499-1499.

Weller PF and Lim K, 1997. Human eosinophil-lymphocyte interaction. *Memorias do ioc* 92, Suppl. II, pp. 173-182.

Wolach B, Falk B, Gavrieli R, Kodesh E and Eliakim A, 2000. Neutrophil function response to aerobic and anaerobic exercise in female judoka and untrained subjects. *Br J Sports Med* 34, pp. 23-27.

Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N et al, 1988. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *Journal of Experimental Medicine* 167, pp. 43-56,

Yamaguchi Y, Suda T, Shizaki H, Miura Y, Hitoshi Y, Tominaga A et al., 1990. Role of IL-5 in IL-2-induced eosinophilia. In vivo and in vitro expression of IL-5 mRNA by IL-2. *The J of Immnunology* 145 (3), pp. 873-877.

Yoko Kano Y, Orihara M, Shiohara T 1998. Cellular and molecular dynamics in exercise-induced urticarial vasculitis lesions. *Arch Dermatol* 134, pp. 62-67.

Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmenn M et al, 2005. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol. *Genomics* 23, pp. 287-294.

Zangrilli JG, Robertson N, Shetty A, Wu J, Hastie A, Fish JE, Litwack G and Peters SP, 2000. Effect of IL-5, glucocorticoid, and Fas ligation on Bcl-2 homologue expression and caspase activation in circulating human eosinophils. *Clin Exp Immunol* 120, pp. 12-21.

Zangrilli JG, 2002. Regulation of eosinophil viability by cytokines. *Am J Respir Cell Mol Biol* 26, pp. 388-390.