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全身性紅斑狼瘡病人多形核嗜中性白血球葡萄糖傳送蛋白 的表現及其功能的研究

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Defective membrane expression of glucose transporter 3 and 6 and associated impaired glucose uptake in polymorphonuclear neutrophils in patients with systemic lupus erythematosus

Abstract

Systemic lupus erythematosus (SLE) is characterized with production of variable autoantibodies and associated immune dysfunction. Patients with SLE are also susceptible to infections, which are partly due to functional impairment of polymorphonuclear neutrophils (PMN). Multiple dysfunction of SLE PMN, including phagocytosis, cytokine production and membrane protein expression etc., was found, but the real pathogenesis is still unclear. Glucose, the primary substrate of energy supply, plays a crucial role in survival and functional maintenance in leukocytes. We determined the membrane expression of glucose transporter (GLUT) by flow cytometry and measured the glucose uptake by use of non-metabolizable glucose analog (NBDG) detected by flow cytometry. The lactate production via anaerobic glycolysis due to ineffective energy supplement in culture supernatant was also measured by ELISA method. The results revealed defective membrane expression of GLUT3 and GLUT6 but not GLUT1 in SLE PMN compared to control PMN, and more significant down-regulation of GLUT expression by LPS or TNF- α stimulation in SLE PMN. Moreover, the glucose uptake was decreased in SLE PMN during 24h culture and poor response to LPS-stimulated glucose uptake as in normal PMN. The increased lactate production reflected the ineffective or insufficiency of glucose supply indirectly in SLE PMN. According to the above findings, defective membrane expression of GLUT and associated impaired glucose uptake resulted in functional impairment in SLE PMN, especially under the stimulation of cytokine or bacterial product LPS. Bioenergetics might play a critical role in the pathogenesis of PMN dysfunction in SLE. But the real mechanism of defective energy supply and the association with underlying autoimmunity need to be further studied.

Keywords: polymorphonuclear neutrophil, glucose transporter, bioenergetics, systemic lupus erythematosus

Introduction

The polymorphonuclear neutrophils (PMN) are characterized as the professional phagocytes. Several interrelated defense mechanisms are deployed that include the

release of highly toxic secretory granule components, activation of respiratory burst, and phagocytosis. Although the respiratory burst activity of PMN (1) and many components of the membrane-bound respiratory burst oxidase for superoxide production (2) has been studied intensively, knowledge of the way that PMN use endogenous and exogenous energy supplies to fuel the respiratory burst is limited (3). Increased superoxide production that is associated with phagocytosis and phorbol myristate acetate (PMA) treatment of neutrophils involves increased glucose-C-1 metabolism via the hexose monophosphate (HMP) shunt (4-6). Chemotactic factor, fMet-Leu-Phe (fMLP) could, but not necessarily, increase the maximal transport velocity of hexoses entering the polymorphonuclear leukocyte via the glucose transporter (7). In the short-term, simple sugar phosphates in neutrophils could provide the energy needed for respiratory burst activity, but in the longer term, stored glycogen is used (8). In addition, PMA activated PMN increased the rate of 2-deoxyglucose (2-DOG) uptake and resulted in increase in transporter affinity for glucose (3). Thereby, neutrophil activation involves increased glucose transport and intrinsic activation of glucose transporter molecules.

Transport of glucose across the plasma membrane is a passive process involving a family of structurally related 'facilitative' glucose transporter molecules, which shift glucose down its concentration gradient without expending energy (9). These transporters are often expressed in a tissue or cell-specific manner, and in some cases, their expression is regulated by extracellular signals such as hormones and growth factors (10). Stress induced by a variety of reagents stimulates glucose transport by translocating transporters from intracellular sites to the plasma membrane (11) or by increasing transporter expression (12). In addition, interleukin-3 (IL-3) and other growth factors stimulate glucose transport in growth factor-dependent cells by increasing the affinity of glucose transporters for glucose without a change in transporter expression or V_{max} (13,14). Frauwirth KA etc. reported that CD28 co-stimulation, acting through phosphatidylinositol 3'-kinase (PI3K) and Akt, is required for T cells to increase their glycolytic rate in response to activation (15). These glucose transport facilitators are about 500 amino acids in length and belong to a growing superfamily of integral membrane glycoproteins with 12 trans-membrane helices. The genes of these carriers have been designated GLUT1 to GLUT9 in the order in which they were identified. The GLUT1, 3, and 4 represent high affinity transport facilitators and GLUT6 expresses on spleen, leukocyte and brain with sugar transport activity (16,17).

Systemic lupus erythematosus (SLE), the archetype of systemic autoimmune disease, comprises a series of immunologic dysfunction among different immune cells, which contributes the clinical susceptibility to infections (18,19). PMN, the first-line

phagocyte of defense barrier, needs the immediate energy supplement such as glucose to support the activation of respiratory burst and phagocytic functions. In spite of multiple functional impairment of PMN in patients with SLE, including reduced phagocytosis (20), the presence of a serum inhibitor for phagocytosis (21,22), decreased nitroblue tetrazolium dye reduction (22) and cytokine production of IL-1, IL-1ra and IL-8 (23,24), the studies of underlying pathogenesis are limited.

Bioenergetics is a relative new field in the biology of immune cells. Growing evidences suggest that glucose is the major energy supply to maintain the function and survival of leukocytes, especially during activation. We want to conduct this study to explore the fuel supply of these defective PMN in SLE and to clarify the possible pathogenesis of the functional impaired SLE PMN.

Results

Decreased membrane expression of glucose transporter 3 and 6 in patients with SLE

The membrane expression of glucose transporter 3 was significantly decreased in patients with SLE ($36.9 \pm 8.2\%$) than that of normal control ($68.9 \pm 5.9\%$) ($N=8$, $P<0.01$) (Fig.1-(A)). The similar tendency was also found in glucose transporter 6 ($N=8$, $84.4 \pm 4.6\%$ in SLE vs. $96.1 \pm 1.1\%$ in control, $P<0.05$). But there was no significant difference in expression of glucose transporter 1 between SLE and normal control. On representative case was showed in Fig.2 (B). The defective expression of certain glucose transporter in SLE PMN with preserved housekeeping glucose transporter 1 might suggest the survival maintained in SLE PMN with functional impairment. In addition, the membrane expression of glucose transporter was down regulated in the presence of IL-8, LPS and TNF- α (Fig.3). The LPS and TNF- α were more powerful than IL-8 as the glucose transporter down regulator. The down-regulation of glucose transporters was more sensitive to the inflammatory cytokines or LPS activation in SLE PMN than that of normal PMN. The experiments were repeated for five times with similar tendency.

Impaired glucose uptake of SLE PMN via non-metabolizable glucose analog (NBDG) determined by flow cytometry

We used isotope-labeled deoxy-glucose (^3H -2-deoxy-D-glucose) to determine the glucose uptake of normal and SLE PMN initially. The glucose uptake was significantly decreased in SLE PMN than that of normal control ($P<0.05$, Fig. 2-(A)). Moreover, this impaired glucose uptake improved partially after control of disease activity but without statistic significance. Further analysis of glucose uptake by use of non-metabolizable glucose analog (NBDG) during different culture period, there was

marked increase in glucose uptake in SLE PMN with active disease during first four hours culture but significant decrease after 24 hours culture (Fig.4). Pre-activated SLE PMN with active glucose uptake should be considered. But PMN from inactive SLE revealed similar result as the control PMN. In addition, the glucose uptake of normal PMN could be enhanced under the activation of LPS during the 4h culture period but the accumulating amount of glucose analog was significantly less in LPS-stimulated PMN than that of control PMN after 24h culture period. Moreover, the PMN from active SLE could not response well to the activation of LPS. Similar experiments were repeated for five times with similar result. According to the above finding, under the stimulation of inflammatory cytokine or bacterial product, the energy supply of PMN was rapidly downhill after exposure to exogenous pathogen in normal PMN.

Increased spontaneous production of lactate in SLE PMN

Lactate, product of anaerobic glycolysis, play as a major short-term and often-initial energy source in many cells. Lactate production was marked increase in the culture supernatants of SLE PMN than that of normal PMN (Fig.5). In addition, this increased lactate production was also found under the stimulation of LPS but not IL-8 in normal PMN but no significant difference in SLE PMN with/without LPS.

Discussion

Systemic lupus erythematosus (SLE) has been regarded as the prototype of systemic autoimmune disease characterized with multiple immune dysfunctions and protean clinical manifestations. Infection is not only the common complication in SLE but also responds to one of the major causes of morbidity and mortality (18,19). In addition to the traditional role of phagocytes as the first line defense barrier against exogenous pathogens, more and more evidence supports that polymorphonuclear neutrophils (PMN) is also crucial to the intact of immune regulation network. Despite multiple functional impairment of PMN in patients with SLE, including reduced phagocytosis (20), the presence of a serum inhibitor for phagocytosis (21,22), decreased nitroblue tetrazolium dye reduction (22) and cytokine production of IL-1, IL-1ra and IL-8 (23,24), the studies of underlying pathogenesis are limited. Several important findings were noted in this study: (a) defective membrane expression of glucose transporter 3 and 6 (GLUT3 and GLUT6) in SLE PMN, which more sensitive to down-regulation under activation by LPS, TNF- α and IL-8, (b) decrease accumulating non-metabolizable glucose analog NBDG in SLE PMN during 24h culture (c) no effective response to LPS enhanced glucose uptake in SLE PMN during first 4h incubation. These results suggested relative energy crisis was present in PMN

from active SLE because glucose is essential to the functional competence of leukocytes, especially during active effectors under stimulation.

PMN, as the professional phagocyte, needs the immediate energy supplement such as glucose to support the activation of respiratory burst and phagocytic functions. Orlinska etc. reported that glucose is required for lipopolysaccharide (LPS)–induced IL-1 β production by monocytes and is the major source of ATP for IL-1 β production (25). Meanwhile, ligands for cell specific receptors such as growth factor preventing apoptosis in non-proliferating cells are to promote nutrient uptake and cellular metabolism. Innumerable studies focused on various cellular mechanisms of specific and non-specific immunity, both involving stimulated leukocytes as key mediators. Therefore, the activation of white blood cells imposes acute energy-metabolic demands (26), which are almost exclusively met by glycolysis (27). Glucose, primary substrate of energy supplement, can either be supplied by uptake from extracellular space or by catabolism of intracellular glycogen. In addition, sufficient cellular glucose transfer is provided by preceding accumulation of glycogen and the glycogen synthesis is further limited by glucose transport efficiency (17). Defective membrane expression of glucose transporter 3 and 6 but preserved GLUT1 elucidated that maintained PMN survival but impaired functions in SLE patients due to ineffective glucose uptake and following glycogen synthesis. The result of glucose uptake by non-metabolizable NBDG further confirmed the deficiency of glucose storage in SLE PMN. The increased production of lactate in SLE PMN culture supernatant gave indirect evidence that energy supplement of SLE PMN was highly inadequate and dependent on less effective anaerobic glycolysis. Therefore, from the point of Bioenergetics, defective GLUT expression result in insufficiency energy supply might contribute the multiple dysfunction of SLE PMN, especially during active state.

In conclusion, bioenergetic defect is one of the contribution factors in the pathogenesis of SLE PMN dysfunction, which might be resulted from defective GLUT3 and GLUT6 membrane expression and associated impaired glucose uptake. But the disease activity *per se*, variable clinical manifestations such as nephritis, leukopenia or infections and treatment in SLE might influence the status of neutrophils. More cases are needed to get more solid result, especially about glucose uptake and lactate production under different stimulation. In addition, the underlying mechanism associated the defective GLUT expression and the relationship with autoantibody production in SLE need to be further clarified.

Fig.1

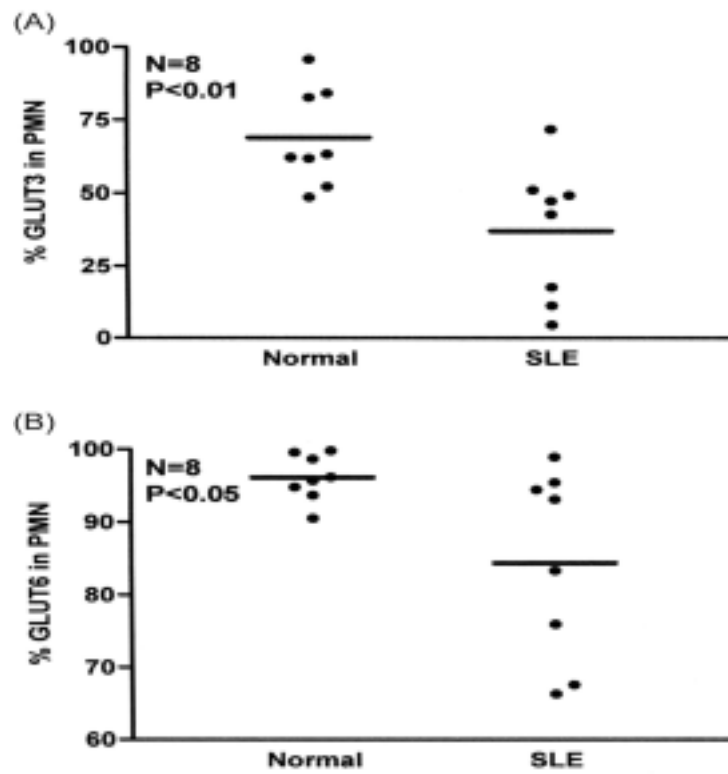


Fig.2

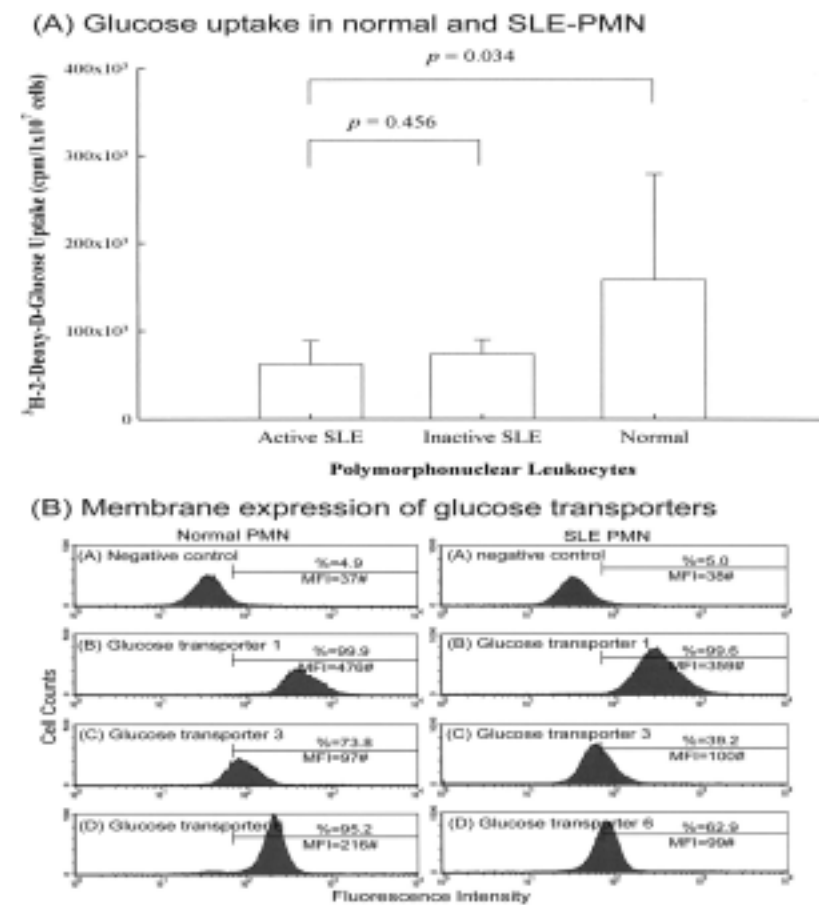


Fig.3

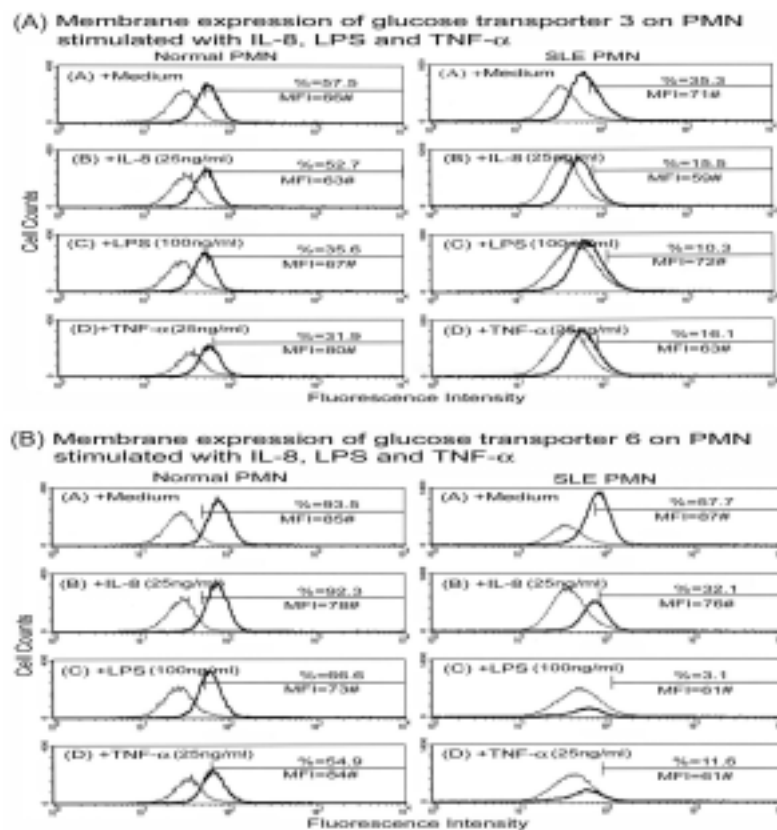


Fig.4

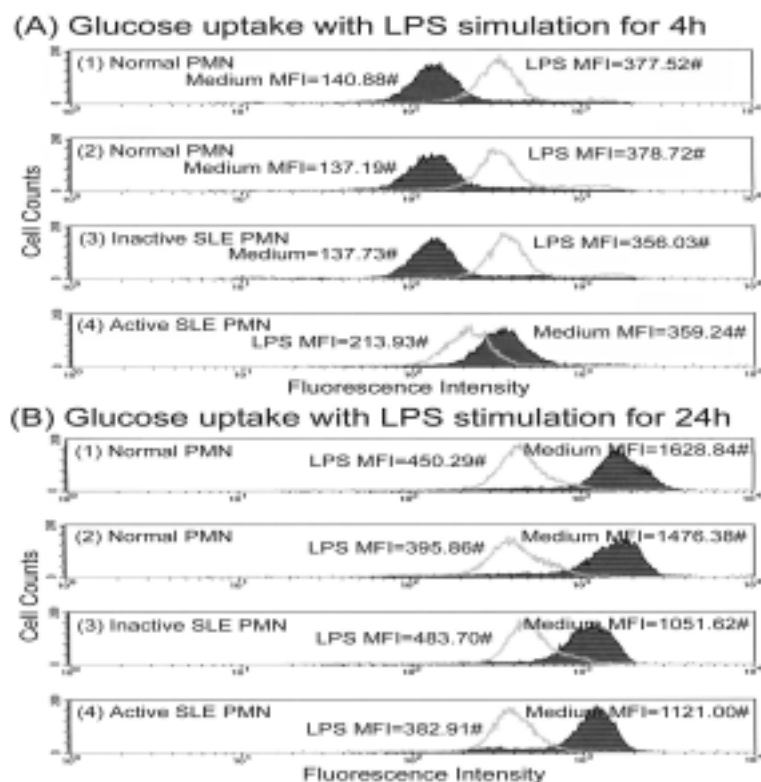


Fig.5

Lactate production by normal and SLE PMN

