

行政院國家科學委員會專題研究計畫 成果報告

全身性紅斑狼瘡病人多形核嗜中性白血球趨化激素受器的 表現及其對趨化激素的反應

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Abnormal modulation of IL-8 receptor CXCR2 leading to functional inertia of polymorphonuclear neutrophils in patients with systemic lupus erythematosus¹

ABSTRACT

Defective phagocytosis, decreased IL-8 production, and hyporesponsiveness to IL-8 stimulation are important factors predisposing systemic lupus patients (SLE) susceptible to bacterial infections. For elucidating the molecular basis of functional inertia in neutrophils of SLE (SLE-PMN), the expression and modulation of IL-8 receptors, and the responsiveness of IL-8 stimulation of these cells were determined. We found the membrane expression of CXCR2, but not CXCR1, was decreased but cytosolic CXCR2 was increased in SLE-PMN compared to normal PMN. However, the binding affinity of the CXCR2 with IL-8 was not different in both PMNs. By contrast, the IL-8-induced CXCR2 down-regulation in SLE-PMN was decreased because of decreased internalization and impaired proteolytic cleavage of the ligand-receptor complexes by the cells. In addition, the expression of CXCR2 in response to genistein, cycloheximide and catalase, but not superoxide dismutase, was also different between the two cells. In conclusion, defective expression of CXCR2 including the hypo-responsiveness and abnormal transport render the functional inertia in SLE-PMN.

RESULTS

Decreased IL-8 production and impaired phagocytosis of SLE-PMN after stimulation

The functional defects of SLE-PMN are demonstrated by decreased IL-8 production (Fig.1-A) and impaired phagocytosis (Fig.1-B) after stimulation with PMN activators including LPS, IL-8, TNF- α and GRO- α compared to normal PMN.

Abnormal expression of IL-8 receptor CXCR2, but not CXCR1, on the cell surface and in the cytosol of SLE-PMN

CXCR1 is a highly specific receptor for IL-8 whereas CXCR2 can bind IL-8 and other ELR(+)-CXC chemokines such as GRO- α , β , and γ with equal high affinity (15,16) on human PMN. We measured both CXCR1 and CXCR2 expression on normal and SLE-PMN and found significant decreased CXCR2, but not CXCR1, expression on SLE-PMN (Fig. 2-A). A representative case was shown in Fig.2-B. The decreased mRNA expression of CXCR2, but not CCR1, was also noted in SLE-PMN as demonstrated in Fig.2-C. On the contrary, the cytosolic expression of CXCR2 in SLE-PMN was more than normal PMN in resting state but was less after IL-8 stimulation (Fig.3-A & B). However, the binding affinity of CXCR2 expressed on normal and PMN-SLE with IL-8 was not different (Fig. 4-A & B). These results

suggest that decreased CXCR2 expression on PMN-SLE, rather than binding affinity, is one of the factors responsible for the hyporesponsiveness to IL-8 stimulation in SLE-PMN. Decreased surface CXCR2 expression on SLE-PMN may be due to defective translocation of the receptors to the surface leading to increased accumulation of CXCR2 in the cytosol of the cells.

Impaired CXCR2 down-regulation of SLE-PMN after reaction with IL-8

The binding of CXCR2 with its ligand, IL-8, induces down-regulation and desensitization of the receptors on PMN (18). Compared to normal PMN, the IL-8-induced down-regulation of CXCR2 in SLE-PMN is remarkably decreased (Fig 5-A). The molecular basis of CXCR2 down-regulation after binding with its ligand may involve at least three mechanisms: (a) endocytosis or internalization of receptors (31). (b) recycling of the internalized receptors (35,36), and (c) proteolytic cleavage of the receptors in the membrane and then are released into the exterior (37). As demonstrated in Fig.3 (A & B) and Fig.5-B, the CXCR2 internalization after IL-8 binding was less in SLE-PMN compared to normal PMN. We compared the amount of soluble CXCR2 (sCXCR2) that represents the proteolytic cleavage products of membrane form CXCR2 in the IL-8-treated PMN cultured supernatants by EIA. As demonstrated in Fig.5-C, a significant decrease of sCXCR2 in IL-8-induced SLE-PMN was noted.

Differential effects of different metabolic inhibitors in CXCR2 surface expression on normal and SLE-PMN

Tyrosine kinase inhibitor genistein, protein synthesis inhibitor cycloheximide, and hydrogen peroxidase inhibitor catalase suppressed normal PMN but conversely enhanced SLE-PMN, surface expression of CXCR2 in resting state. The same tendency was also noted in IL-8-mediated CXCR2 down-regulation in both normal and SLE-PMN. By contrast, superoxide anion radical inhibitor SOD enhanced the spontaneous and IL-8 induced CXCR2 expression in both PMN. These results may reflect differential modulation pathways in signaling, protein synthesis and oxidative stress in CXCR2 expression on SLE-PMN.

DISCUSSION

A number of immune functional defects have been found in patients with SLE including decreased production of IL-1 and IL-2 (38,39), depressed phagocyte and cell-mediated immunity (4), and hyporesponsiveness of MNC to mitogens (4, 40), antigens (41), allogeneic cells (42), and autologous MLR (43). Among these defective phagocytosis (4), decreased spontaneous and LPS-stimulated IL-8 production, and hyporesponsiveness to IL-8 stimulation (7) of PMN are the major functional inertia predisposing patients with SLE susceptible to bacterial infection. In the present study, we intend to elucidate the molecular basis of functional inertia of SLE-PMN by

detection of the expression and modulation of IL-8 receptors in SLE-PMN that related to IL-8 responsiveness. Several original findings are derived from these studies: (i) Decrease expression of IL-8 receptor CXCR2, but not CXCR1, on SLE-PMN whereas the binding affinity with IL-8 is not impaired. (ii) Abnormal modulation of CXCR2 in SLE-PMN in that increased spontaneous cytosolic accumulation and decreased down-modulation and after binding with IL-8. It is deduced that the underlying pathophysiological basis for the pleiotropic defects of SLE-PMN originated from the PMN in pre-excitation and then in exhausted state *in vivo* activated by proinflammatory cytokines, chemokines, immune complexes, oxygen metabolites or inflammatory mediators in the SLE patients.

Different from CXCR1 that has highly specific affinity for IL-8, CXCR2 can bind with IL-8 and the other ELR (+)-CXC chemokines with equal high affinity. Accordingly, CXCR2 shows much more pleiotropism for inflammatory responses than CXCR1. Clinically, CXCR1 acts as the single dominant CXC chemokine receptor in patients with sepsis whereas CXCR2 expression was down-regulated by 50% for attenuating adverse inflammation in such a complex milieu of multiple CXC chemokines (44). In addition, CXCR2 plays a crucial role in the pathogenesis of PMN recruitment in helminth-mediated keratitis (45) and ventilator-induced lung injury rather than CXCR1 (46). Physiologically, the modulation of CXCR2 on PMN surface can be down-regulated by many factors including TNF- α (47,48), bacterial endotoxin (49), and CXCR2-ligands (18,20). After binding with these factors, CXCR2 is down regulated through either mechanism of clathrin-mediated endocytosis (31), actin filament-mediated intracellular trafficking (35-37), or tyrosine kinase-dependent mechanism (49). Studies have suggested that this rapid ligand induced CXCR2 down regulation is consistent with the physiological function of chemotactic factors that is concentration-dependent. In low concentration, the effector cells are response rapidly and migrate toward the center of chemokine production. Along the way of migration the concentration of chemokine may increase gradually. Down-regulation of chemokine receptors would occur for preventing the over migration and stop the effector cells in the site of inflammation. A number of interesting findings regarding the abnormal CXCR2 modulation in SLE-PMN were found in the present study: (i) Decrease surface expression with increase cytosolic accumulation of CXCR2. (ii) Defective down-regulation of CXCR2 after binding with IL-8. We also found the decreased IL-8-induced CXCR2 down-regulation in SLE-PMN is the result of decreased internalization of CXCR2-IL-8 complexes and impaired proteolytic cleavage of the ligand-receptor complexes in the surface membrane. It is conceivable that CXCR2 contains internalization motifs LLKIL (36). The CXCR1 internalization is negatively controlled by induction of signaling events, as indicated by the

promotion of CXCR2 internalization following exposure to wortmannin, a potent inhibitor of phosphatidylinositol (PI) 3 kinase and PI4 kinase (31,35,36). Following internalization and removal of the ligands from the vicinity of the cells, the receptors are recycled back to the plasma membrane in an unphosphorylated form, ready to be restimulated by their corresponding ligands (31). Zaslaver et al. (35) and Matityahu et al. (36) demonstrated that actin filaments are involved in the regulation of trafficking of CXCR1 and CXCR2. Furthermore, rab 11⁺-endosomes participate in the intracellular trafficking of CXCR2 through the endocytic pathway and eventually allow its recycling back to the plasma membrane (31,35,36). We did not study the CXCR2 recycling after binding with IL-8 in either normal or SLE-PMN. Whether CXCR2 recycling in SLE-PMN is abnormal or not is not clear and needs further investigations.

The expression of CXCR2 on normal and SLE-PMN was differentially affected by different protein inhibitors. We found tyrosine kinase could suppress CXCR2 expression on normal PMN. This finding is quite similar to the result of Khandaker et al. (49) that genistein attenuated the LPS-mediated down-regulation of CXCR1 and CXCR2. We also noted that protein synthesis and hydrogen peroxide suppressed whereas superoxide anion radicals enhanced the CXCR2 expression on normal PMN. In contrast, the reverse effects were noted in SLE-PMN except superoxide anion radicals. These results indicate that different regulatory mechanisms of CXCR2 expression, ligand-induced down-regulation, and metabolism pathways may exist in normal and SLE-PMN. The pre-excited SLE-PMN seemed to exhibit particular cell biology different from normal PMN. However, The real cause for the different modulation pathway of CXCR2 in normal and SLE-PMN need further investigation.

In conclusion, we are the first to elucidate the pathophysiological basis of functional inertia of SLE-PMN particularly on the IL-8 hyporesponsiveness that responsible for susceptible to bacterial infection. We found defective expression and abnormal modulation of CXCR2 on SLE-PMN are the contributory factors for the functional impairment in these cells.

Fig.1

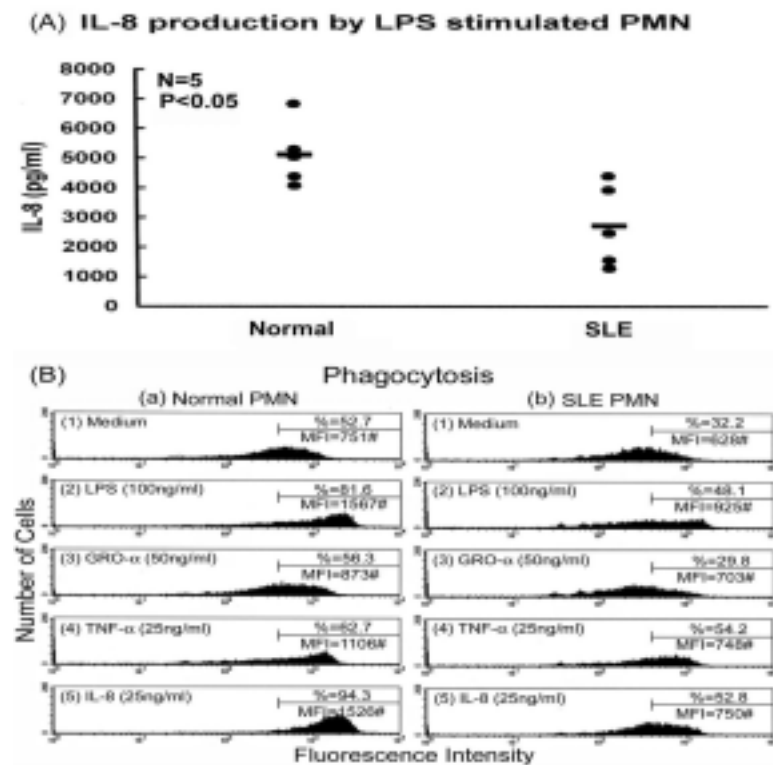


Fig.2

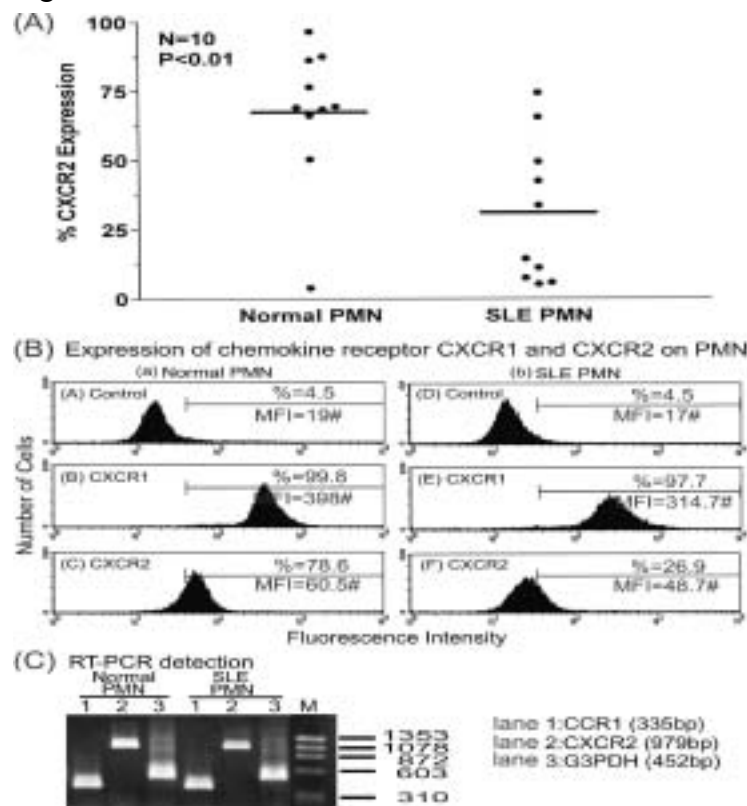
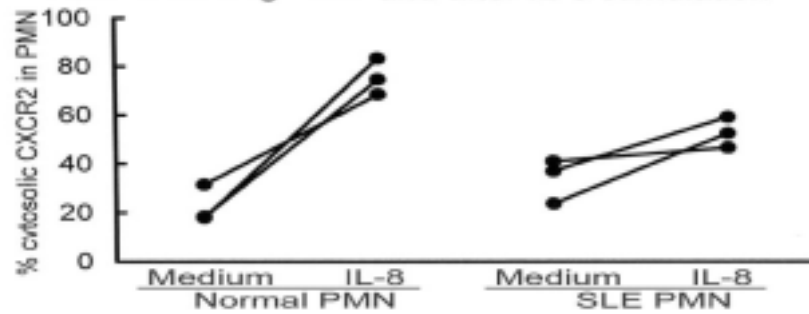


Fig.3

(A) Cytosolic expression of CXCR2 in normal and SLE PMN in resting state and after IL-8 stimulation



(B) A typical case demonstrating cytosolic expression of CXCR2 in PMN after incubation with medium or IL-8

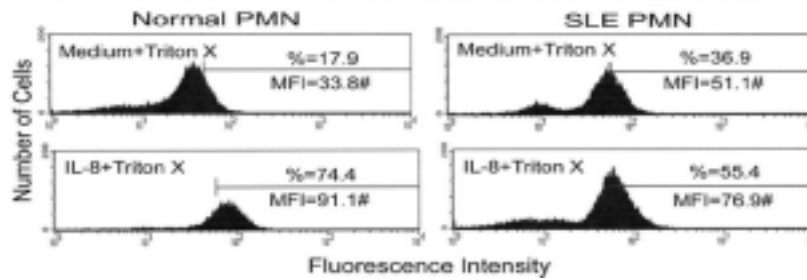
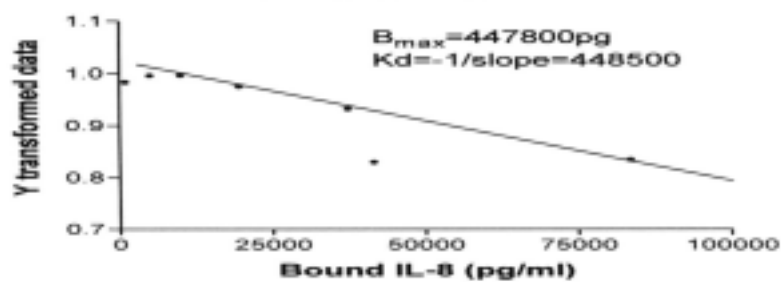


Fig.4

(A) Binding affinity of IL-8 with CXCR2 on normal PMN



(B) Binding affinity of IL-8 with CXCR2 on SLE PMN

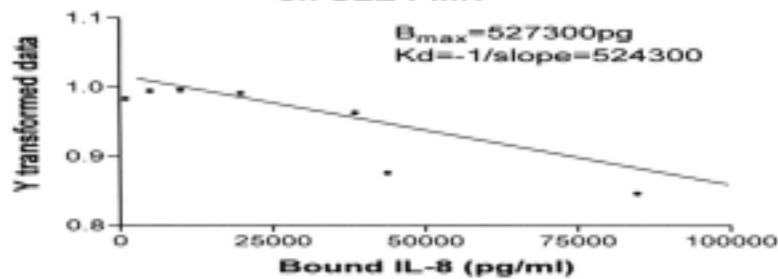
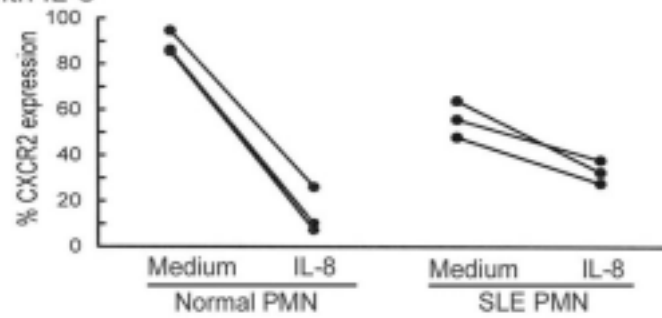
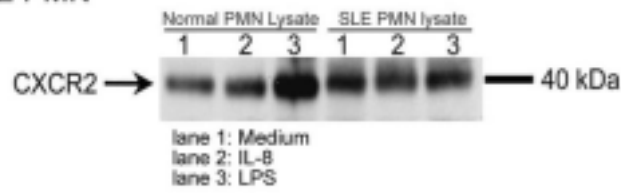


Fig.5

(A) Down-regulation of membrane CXCR2 on PMN after binding with IL-8



(B) Internalization of CXCR2 by IL-8 and LPS in normal and SLE PMN



(C) Soluble CXCR2 in cultured supernatant of PMN+IL-8

