Impaired Immunity and Oxidative Stress in End Stage Renal Failure Patients

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혈액투석중인 말기 신부전환자에서 면역기능의 이상과 산화스트레스

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〈Abstract I〉

ESRD is associated with increased propensity to infections, diminished response to vaccination, impaired cell-mediated immunity and reduced CD4+/CD8+ T lymphocyte ratio. Four subsets of CD4+ and CD8+ T cells have been recently identified; naïve cells (as-yet uncommitted), central memory cells (previously programmed), CD45RA-positive and CD45RA-negative effector memory cells (programmed to perform specific effector functions). The effect of ESRD on subpopulations of T lymphocytes is unclear and was studied here. Twenty one hemodialysis patients and 21 age-matched controls were studied. Pre- and post-dialysis blood samples were obtained and analyzed by 3-color flow cytometry. CD4+/CD8+ ratio, and the numbers of the naïve and central memory CD4+ and CD8+ T cells were significantly reduced, whereas, the numbers of effector memory CD4+ and CD8+ T cells were unchanged in the ESRD group. The reduction of the naïve and central memory T cell counts in the ESRD group was associated with increased apoptosis of these cells. Negative correlations were found between severity of azotemia, oxidative stress and hyperphosphatemia with the number of naïve T cells. Comparison of diabetic with non-diabetic ESRD patients revealed higher numbers of total CD8+ cells and effector memory CD8+ T cells in the diabetic group. Dialysis did not significantly change the naïve and central memory CD4+ or CD8+ cell counts, but significantly lowered CD8+ effector memory cell count. Thus, ESRD results in increased apoptosis and diminished populations of naïve and central memory T lymphocytes. This phenomenon may, in part, contribute to the impaired immune response in this population.

〈Abstract II〉

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Oxidative stress and inflammation are common features and major mediators of atherosclerosis in end stage renal disease (ESRD). Available evidence for oxidative stress in ESRD is indirect and based on accumulation of byproducts of interactions of reactive oxygen species (ROS) with various molecules. Inflammation is a major cause of oxidative stress. To explore the direct link between oxidative stress and inflammation in ESRD we studied leukocyte integrin expression and ROS production in 18 ESRD patients and 18 controls. ESRD patients showed elevated plasma malondialdehyde (MDA) and increased superoxide and H_2O_2 production by granulocytes and monocytes before dialysis. Hemodialysis resulted in a further rises in plasma MDA and H_2O_2 production by granulocytes and monocytes. Integrin (CD11b, CD18) expressions on granulocytes and monocytes were significantly increased in ESRD patients. Granularity of granulocytes was significantly reduced before dialysis and declined further after dialysis. The magnitude of ROS production by granulocytes and monocytes significantly correlated with the degree of azotemia, plasma ferritin and PTH levels. Thus, present study provides direct evidence of spontaneous leukocyte activation and increased ROS generation (hence the link between oxidative stress and inflammation) in ESRD patients.

Impaired immunity in ESRD Patients

While bacterial infections have diminished as a cause of death in the general population, they constitute the second most common cause of death in the end stage renal disease (ESRD) population. This is thought to be largely due to the impaired host immune response in uremia. The reported immunological abnormalities in ESRD patients include : decreased granulocyte and monocyte/macrophage phagocytic function, defective antigen presentation by monocyte/macrophages, reduced antibody production by B lymphocytes and impaired T cell-mediated immunity. The exact mechanisms responsible for these derangements are not fully understood. So we evaluate several immunologic abnormality that might explain immunologic abnormality in ESRD patients.

1. Naïve and Central Memory T cell Lymphopenia in End-Stage Renal Disease

T lymphocytes play a central role in generation of the adaptive immune response. In the presence of infection, naïve (pre-immune) T cells recognize epitopes of the structural molecules expressed by the invading microbe. This leads to activation, massive expansion and differentiation of these cells into two types of antigen-experienced lymphocyte subsets, short-lived effector T cells and long-lived memory T cells. Once, re-challenged with the same antigen, the long-lived memory T cells can elicit the full immunologic response rapidly. Recently, several studies have identified two major subsets within the memory cell population, namely, central memory (CM) and effector memory (EM) T lymphocytes. The effector memory T cells have been further divided

into two subpopulations, the CD45RA-negative effector memory and CD45RA-positive effector memory (TEMRA) T cells (Fig. 1). The effector memory cells exert effector functions at the sites of inflammation while the naïve and central memory cells express homing receptors which allow them to lodge in the secondary lymphoid organs. A limited number of studies have examined the distribution and function of T lymphocytes in the ESRD patients. However, little information is available on the effect of ESRD on the naïve and memory T lymphocytes subsets. Therefore, we evaluated T lymphocyte subsets pre- and post-hemodialysis in a group of ESRD patients and compared the results with those obtained in a group of age-matched control individuals. CD4+/CD8+ ratio, and the numbers of the naïve and central memory CD4+ and CD8+ T cells were significantly reduced, whereas, the numbers of effector memory CD4+ and CD8+ T cells were

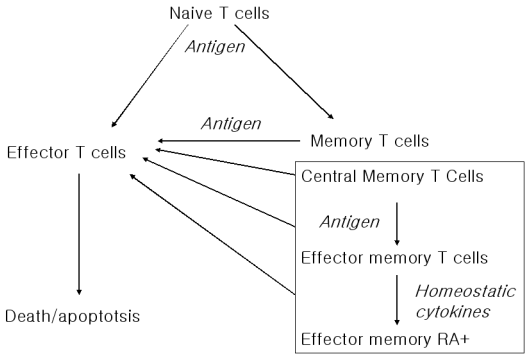

Fig. 1. *A linear model of central and effector T cell memory generation in primary response to infection*

unchanged in the ESRD group (Fig. 2, 3). The reduction of the naïve and central memory T cell counts in the ESRD group was associated with increased apoptosis of these cells. Negative correlations were found between severity of azotemia, oxidative stress and hyperphosphatemia with the number of naïve T cells. Comparison of di-

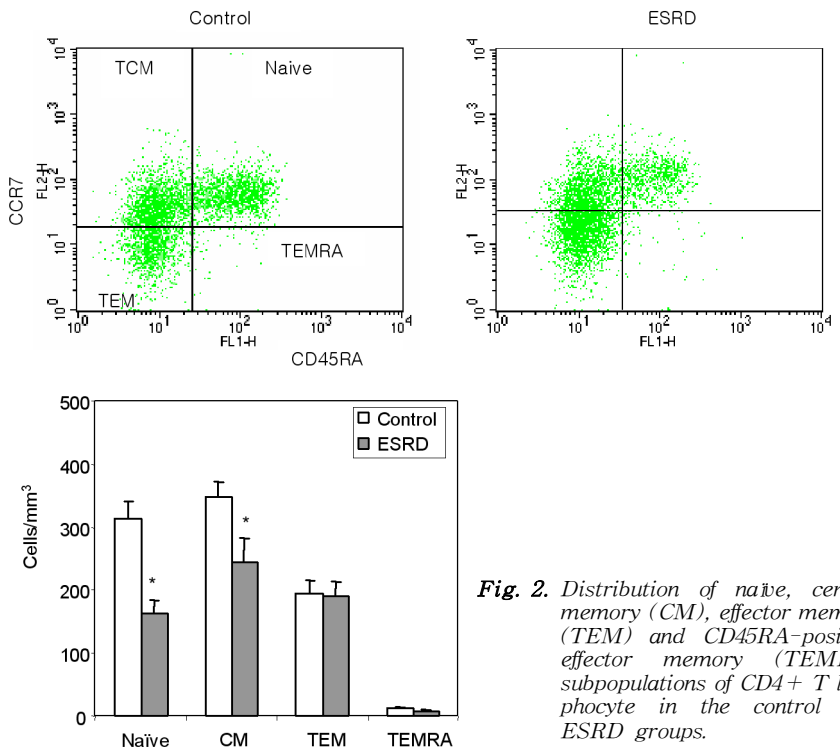

Fig. 2. *Distribution of naïve, central memory (CM), effector memory (TEM) and CD45RA-positive effector memory (TEMRA) subpopulations of CD4+ T lymphocyte in the control and ESRD groups.*

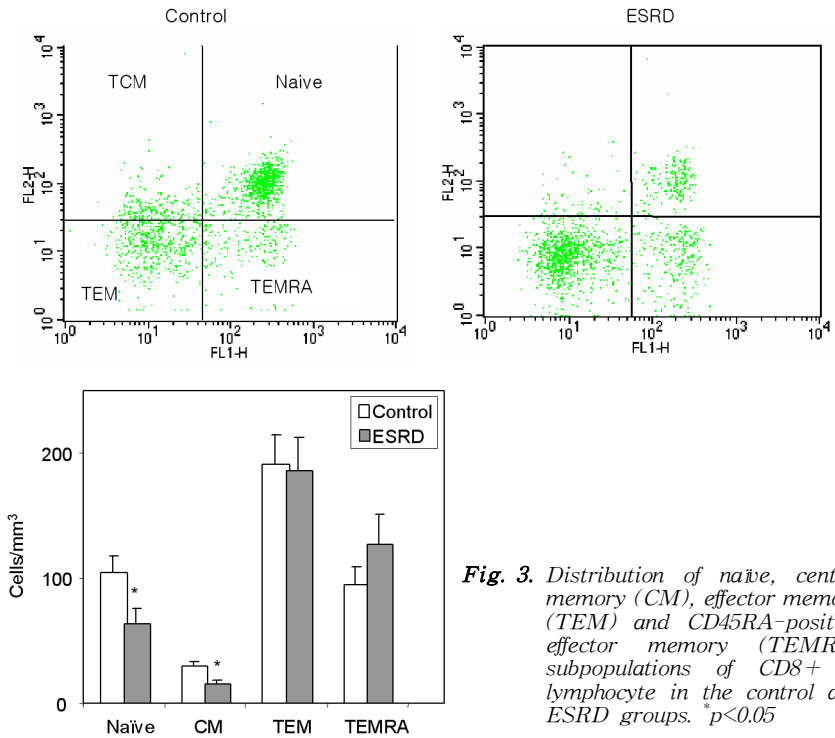

Fig. 3. *Distribution of naïve, central memory (CM), effector memory (TEM) and CD45RA-positive effector memory (TEMRA) subpopulations of CD8+ T lymphocyte in the control and ESRD groups. *p<0.05*

abetic with non-diabetic ESRD patients revealed higher numbers of total CD8+ cells and effector memory CD8+ T cells in the diabetic group. Dialysis did not significantly change the naïve and central memory CD4+ or CD8+ cell counts, but significantly lowered CD8+ effector memory cell count. Thus, ESRD results in increased apoptosis and diminished populations of naïve and central memory T lymphocytes. This phenomenon may, in part, contribute to the impaired immune response in this population.

2. Effect of End Stage Renal Disease (ESRD) and Hemodialysis (HD) on Toll-like Receptor (TLR) Expression and Activities

ESRD is associated with inflammation, impaired immunity and increased susceptibility to bacterial infections. HD with high-flux dialyzers frequently results in the influx of endotoxin fragments from dialysate to blood compartment. TLRs consist of a group of receptors which recognize structural patterns of molecules expressed on infectious microorganisms. TLRs are known to play an essential role in first line of the innate immune process which recognize foreign protein and invading various microorganisms. TLRs are known to be expressed cell surface of granulocytes, monocytes and macrophages of peripheral bloods. This study tested the hypothesis that increased susceptibility to infections or alternatively the prevailing inflammatory state in the ESRD may be due, in part, to defective expression or funtion of the TLRs. 21 ESRD patients and age-matched controls were studied. Blood samples were obtained before and after HD and processed for TLR2 and TLR4 expressions in circulating granulocytes and monocytes by flow cytometry. In addition, blood samples were incubated with lipopolysaccharide (LPS, TLR4 ligand)

and peptidoglycan (TLR2 ligand) for 24 hours to assess TNF-α, IL-1 and IL-6 production. TLR2 and TLR4 expressions in granulocytes and monocytes of ESRD patients were not significantly different from those of the controls. Likewise 3-hour HD procedure did not significantly change expression of TLRs. Stimulation with LPS resulted in a significantly higher IL-6 and TNF-α, production in the ESRD patients than in the controls ($p<0.05$). However, response to stimulation with peptidoglycan was similar in the two groups. Thus, TLR2 and TLR4 expression in peripheral granulocytes and monocytes are not significantly altered in stable ESRD patients treated with chronic HD. However, TLR4 activation by LPS results in a supernormal production of pro-inflammatory cytokines in ESRD patients. This may contribute to the pathogenesis of inflammatory state and its untoward consequences in HD-treated ESRD patients. The mechanism for the exaggerated response to LPS stimulation is uncertain and awaits further investigation.

Oxidative Stress and Inflammation in End Stage Renal Disease (ESRD) Patients

Oxidative stress is a common feature and a major cause of cardiovascular and other complications of end stage renal disease (ESRD). While the precise mechanisms of oxidative stress and inflammation in ESRD have not been definitely elucidated, a number of factors appear to be involved. These include uremic toxins, rennin-angiotensin system, hypertension, underlying disease, infection, iron overload, antioxidant deficiency and dialysis procedures among others.

The available evidence for presence of oxidative stress in patients with ESRD is based largely on increased plasma concentration of stable byproducts of interaction of reactive oxygen species (ROS) with various molecules such as lipids/lipoproteins, proteins, thiols, carbohydrates and nucleic acids. While highly suggestive, these findings represent indirect evidence for oxidative stress in this population. The indirect evidence of oxidative stress in patients with ESRD is frequently accompanied by elevated levels of markers/mediators of inflammation. It is of note that oxidative stress and inflammation are intimately inter-related as each recruits and amplifies the other to create a vicious cycle. For instance, oxidative stress can provoke inflammation by activating nuclear transcription factor kB (NFkB) and consequent generation of pro-inflammatory cytokines and chemokines. Production and release of reactive oxygen, chlorine and nitrogen species by the activated immune cells, inherent to inflammation, in turn, promotes oxidative stress.

As noted above, there is ample indirect evidence for presence of oxidative stress and inflammation in patients with ESRD. However, to our knowledge production of reactive oxygen species (ROS) has not been directly demonstrated in ESRD patients. Therefore, the present study was designed to test the hypothesis that circulating leukocytes participate in production of reactive oxygen species in the uremic patients. The study further sought to examine the effect of hemodialysis on production of ROS by circulating leukocytes. ESRD patients showed elevated plasma malondialdehyde (MDA) and increased superoxide and H_2O_2 production by granulocytes and monocytes before dialysis. Hemodialysis resulted in a further rises in plasma MDA and H_2O_2 production by granulocytes and monocytes. Integrin Mac-1 (CD11b and CD18) expressions on granulocytes and monocytes were significantly increased in ESRD patients. Granularity of granulocytes was significantly reduced before dialysis and declined further after dialysis. The magnitude of ROS production by granulocytes and monocytes significantly correlated with

the degree of azotemia, plasma ferritin and PTH levels. Thus, present study provides direct evidence of spontaneous leukocyte activation and increased ROS generation (hence the link between oxidative stress and inflammation) in ESRD patients.

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