Journal of Korean Medical Science
Vol. 7, No. 4, 343-348 December 1992

Studies on the Role of Interleukin-4 and Fc ε RII in the Pathogenesis of Minimal Change Nephrotic Syndrome

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Childhood minimal change nephrotic syndrome (MCNS) has often been associated with allergic symptoms such as urticaria, bronchial asthma, atopic dermatitis, allergic rhinitis and elevated IgE levels and referred to involve immune dysfunction. Fc ε RII is known to be involved in IgE production and response. Interleukin-4 is being recognized as a major cytokine up-regulating IgE production. Hence the present study is aimed at investigating the role of interleukin-4 and Fc ε RII in the pathogenesis of MCNS. IgE was measured by ELISA. Fc ε RII was analyzed by fluorescence activated cell scanner (FACscan) by double antibody staining with anti Leu16-FITC and anti Leu20-PE. Soluble IgE receptor was measured by ELISA using anti CD23 antibody (3-5-14). Interleukin-4 activities were measured by CD23 expression on purified human tonsillar B cells. Serum IgE levels were significantly higher in MCNS (1,507 ± 680 IU/dl) than in normal controls (123 ± 99.2 IU/dl). A significantly higher expression of membrane Fc ε RII was noted for MCNS (41 ± 12%) than that in normal controls (18 ± 6.2%) ($p<0.001$). Soluble CD23 levels were also significantly higher in MCNS (198 ± 39.3%) than in normal controls (153 ± 13.4) ($p<0.01$). Interleukin-4 activity in sera of MCNS (12U/ml) was also significantly higher than normal controls (4.5U/ml). These results indicate that increased production of Fc ε RII and interleukin-4 may play an important role in the pathogenesis of MCNS.

Key Words: *Minimal Change Nephrotic Syndrome, Fc ε RII, Interleukin-4*

INTRODUCTION

Minimal change nephrotic syndrome is the most common type of childhood nephrotic syndrome, and is often associated with allergies such as atopic dermatitis, bronchial asthma, allergic rhinitis and elevated serum IgE levels. Relapse is frequently preceded by immunologic stimuli such as upper respiratory illness, contact dermatitis, or bee stings. Although the pathogenesis of MCNS has not been clearly defined, current hypothesis favors a disorder in T cell dysfunction as proposed by Shalhoub (1974). On contact with a specific stimulus, sensitized lymphocytes secrete a number of highly active lymphokines. It is postulated that these lymphokines alter the glomerular anionic sites, resulting in albuminuria of nephrotic state. (Moorthy et al., 1979; Fodor et al., 1982). A variety of lymphokines have been identified in patients with MCNS. Eyres et al. (1976), using a leucocyte migration test, demonstrated that nephrotic syndrome patients have evidence of T cell sensitization. Lagrue et al. (1975) have identified the presence of lymphokine in the supernatant of lymphocytes taken from MCNS patients. Schnaper et al. (1984) and Schnaper and Aune (1985) have identified another lymphokine in MCNS which they named soluble immune response suppressor (SIRS). A prominent role has been proposed for vascular permeability factor (VPF), a lymphokine produced

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by Con A-activated patient lymphocytes which mediates local diffusion of proteins out of capillary beds. Recently VPF has been found in supernatants of cells which were not treated with Con A, indicating that lymphocytes may produce it in vivo. However transcapillary protein leakage induced by VPF is not selective. And also VPF has been found in other glomerular diseases such as IgA nephropathy (Boulton-Jones et al., 1983; Tomizawa et al., 1985; Bakker et al., 1986; Yoshizawa et al., 1989).

Recently Nakabayashi et al. (1991) identified other cytokines (Mw 6,000 & 14,000) which are found in the nephrotic state of MCNS and not found in the remission state of MCNS and other glomerulonephritis such as IgA nephropathy and membranous glomerulonephritis. Also a negligible amounts of the above cytokines are detected in membranoproliferative glomerulonephritis.

Recent studies show that interleukin-4 is a major cytokine that is closely related to allergy, and that it up-regulates Fc ϵ RII and IgE (Snapper et al., 1988; Defrance et al., 1987). Fc ϵ RII is involved in IgE production and response. Thus we investigated the role of interleukin-4 and low affinity IgE receptor (Fc ϵ RII) in the pathogenesis of minimal change nephrotic syndrome.

MATERIALS AND METHODS

Materials

MCNS was diagnosed from clinical and pathologic findings by renal biopsy. All biopsy samples were studied by light, immunofluorescence and electron microscopy. We measured serum IgE levels in 8 MCNS and in 8 normal controls, membrane Fc ϵ RII in 20 (17 males, 3 females) MCNS patients and in 12 normal controls, and serum soluble CD23 levels in 10 MCNS and in 8 normal controls. All patients with minimal change nephrotic syndrome were admitted to the Department of Pediatrics, Kyung Hee University Hospital between 1988 and 1991. Tonsils were obtained from the Otolaryngologic department.

Serum IgE level

Serum IgE levels was analyzed by ELISA, coated with monoclonal anti human IgE HP6029 (1:100).

Preparation of B cells and B cell culture

Nephrotic B cells were prepared from 20ml of heparinized blood from nephrotic state of MCNS patients. Fresh human B cells were prepared from tonsils by Ficoll-Hypaque density gradient centrifugation and rosetting twice with AET-treated sheep RBC. After removing adherent cells, 95% purity of B-cells was obtained as confirmed by anti Leu16 (Becton Dickinson) and anti Leu4 (Becton Dickinson) staining. Purified B cells were cultured in RPMI media (GIBCO) containing 10% FBS (Hyclone). Recombinant human IL-4 (Genzyme, 5 to 200 U/ml) was added to the tonsils at various times and cultured for 2 to 3 days.

mCD23 (Fc ϵ RII) measurement

mCD23 expression on cultured B-cells (1×10^6 cells) was analyzed by double antibody staining with 10μl of anti Leu20 (CD23)-PE (Becton Dickinson) in HBSS containing 3% FBS and 0.1% NaN_3 for 30 minutes at 4°C, using a fluorescence activated cell scanner (FACscan, Becton Dickinson). mCD23 positivity among B-cells was expressed as

$$\frac{\text{CD23 positive B-cells}}{\text{total B-cells}} \times 100$$

Soluble CD23 (sCD23) ELISA

Levels of soluble CD23 in the sera of MCNS and normal controls were analyzed by ELISA. An ELISA-plate (Nunc, Denmark) coated with 10μg/ml of monoclonal 3-5-14 (CD23 antibody, Dr. Kishimoto, Osaka university) was blocked with PBS containing 1% BSA and 0.005% Tween 20. After incubation with samples at 25°C for 2hr, the plate was washed and incubated with 1:1,600 diluent of alkaline phosphatase-conjugated MHM6 Ab (CD23 antibody, Dr. Kishimoto). The enzyme reaction was developed by adding p-nitrophenyl phosphate (Sigma) and the optical density was measured using a Micro ELISA autoreader (MR700, Dynatech, U.S.A.).

IL-4 activities

Fresh B cells from human tonsils were used as marker cells for interleukin-4 activity.

Statistical Analysis

Statistical analysis was performed using student t-test. All values are given as mean $\pm$ one standard deviation. p values less than 0.05 were considered significant.

RESULTS

Serum IgE level

Childhood minimal change nephrotic syndrome is often associated with allergy. Elevated serum IgE has been frequently observed in these patients. We meas-

ured serum IgE levels in nephrotic stage of MCNS and in normal controls (Fig. 1), this revealed 1,507±680 (IU) in the MCNS group and 123±99.2 (IU) in the control group ($p<0.001$). Hence, the present study is aimed at investigating the role of Fc ϵ RII and IL-4 in MCNS.

IgE receptor (membrane CD23) expression on MCNS B cells

CD23 is a B-cell differentiation specific antigen with a molecular weight of 45KD. Its proteolytic cleavage product, soluble CD23, has been identified. Both of these molecules are thought to play a pivotal role in the control of IgE synthesis and IgE responses. It has been demonstrated that IL-4 induces a high rate of IgE production by normal human B-cell under this condition, CD23 expression on B-cells is required for IgE production, and monoclonal anti CD23 antibody specifically blocks IL-4 induced IgE production.

In this study, membrane CD23 was measured in the B-cells of 20 MCNS patients and in 12 normal controls, by double staining with anti Leu 16-FITC and anti Leu 20-PE, and analyzed was in by FACscan analysis. Fig. 2 shows FACscan analysis of IgE receptor expression in B-cells. Panel 2 indicates CD23 positive cells among B-cells, which is 17% in a normal control (Fig. 2A), and shows 47% (Fig. 2B) and 54% (Fig. 2C), in 2 cases of MCNS. In the 20 MCNS group CD23 expressions were 41±12(%) and in normal control group 18±6.2(%) (Fig. 3) ($P<0.001$).

Soluble CD23 level

Two kinds of IgE receptors are identified, one is membrane CD23 on B-cells and the other is soluble

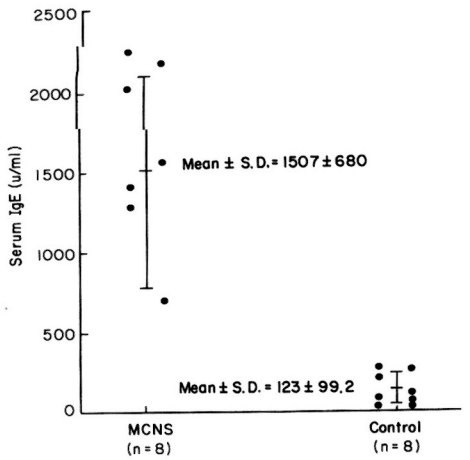


Fig. 1. Serum IgE level in MCNS and normal controls. ($p<0.001$)

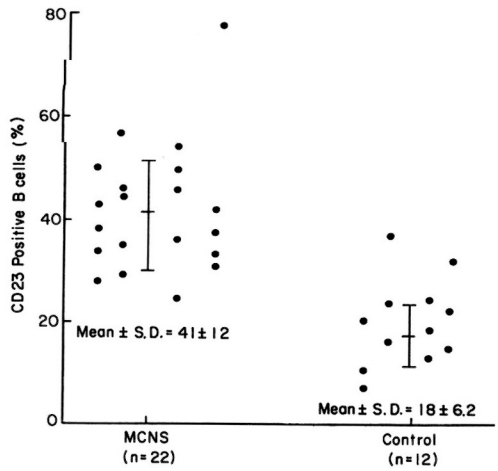
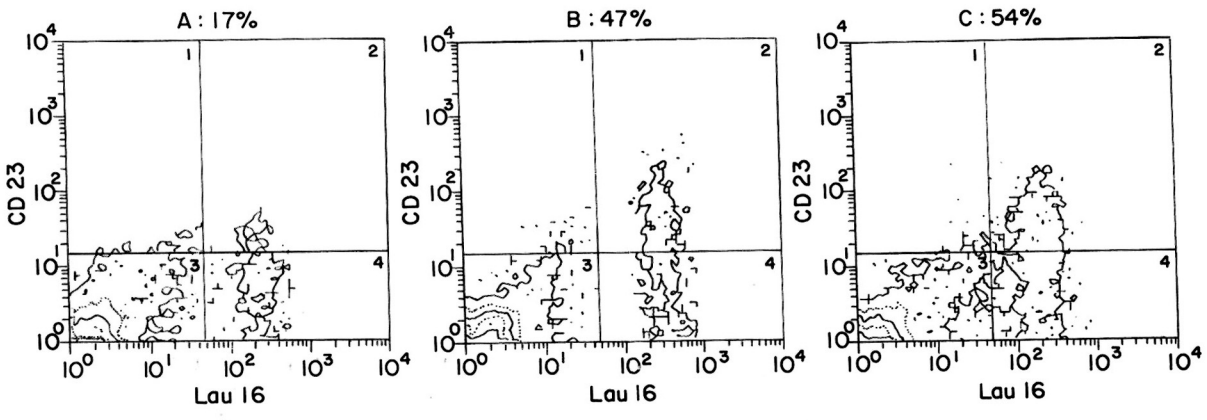


Fig. 3. IgE receptor (CD23) expression on B cells in MCNS and normal controls. ($p<0.001$)



A : Normal control B : MCNS C : MCNS

Fig. 2. FACScan analysis of IgE receptor (CD23) expression on B cells.

CD23, which is shedded from B-cells by enzymatic cleavage. Soluble CD23 itself could combine IgE, and also works as a B-cell growth factor and as a regulator of IgE production. Soluble CD23 is a proteolytic cleavage product from membrane CD23. It is also thought to play a pivotal role in the control of IgE synthesis and IgE responses, does as membrane CD23. We measured soluble CD23 using monoclonal anti CD23 antibody (3-5-14) by ELISA (Fig. 4).

Soluble CD23 levels were 198±39.3U/ml in the 10 MCNS group and 153.7±13.4U/ml in the 8 normal control group ($P < 0.01$)

IL-4 activities in MCNS

IL-4 induces a high rate of IgE production by normal human B cells in a dose dependent manner, however CD23 expression on B cells is mandatory for IgE production, and monoclonal anti CD23 antibody specifically blocks IL-4 induced IgE production. By adding recombinant human IL-4 onto fresh human tonsillar B-cells and culturing for 24hrs, CD23 expression was increased in a dose dependent manner, 8.1% increas was obtained by adding 5U/ml IL-4, 27.7% by 10U/ml IL-4 and 47.3% by 100U/ml IL-4 (Fig. 5).

We measured serum IL-4 activities by CD23 expression as following. We added various concentrations of sera onto purified human tonsillar B cells and cultured for 24 hrs, and then analyzed CD23. By calculating CD23 expression, IL-4 activities were 12U/ml in MCNS patients and 4.5U/ml in normal controls (Fig. 6).

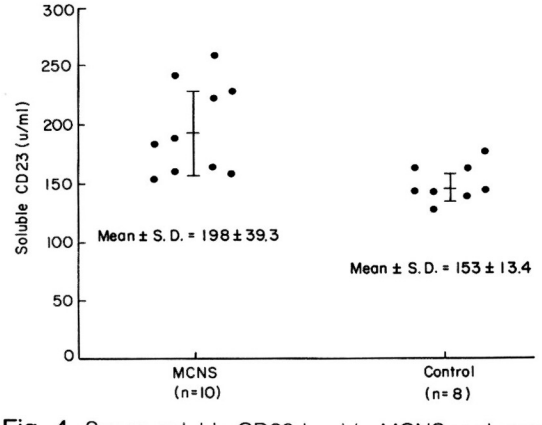


Fig. 4. Serum soluble CD23 level in MCNS and normal controls ($p < 0.01$)

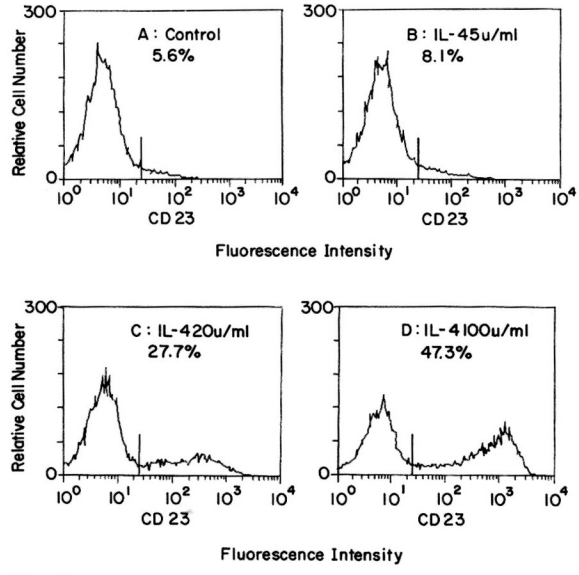


Fig. 5. Patterns of IgE receptor (CD23) expression on tonsillar B cells by IL-4.

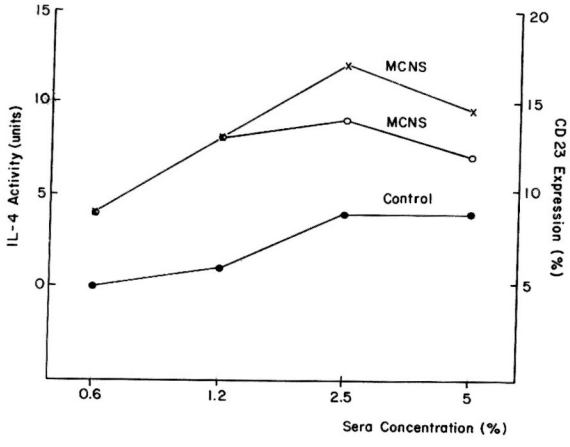


Fig. 6. IL-4 activities in sera of MCNS and normal control.

DISCUSSION

Since Shalhoub (1974) proposed that MCNS represents a generalized disorder of the immune system which has a renal manifestation rather than a specific disease of the kidney, significant progress has been made in research into the mechanism and pathogenesis of MCNS. On contact with a specific antigen, sensitized lymphocytes elaborate a number of highly active lymphokines. It is postulated that lymphokines lead to alterations in the glomerular anionic sites, resulting in the albuminuria of nephrotic state. A variety of lymphokines have been identified in patients with MCNS (Ooi et al., 1974; Barnes et al., 1984).

MCNS is frequently associated with allergic symptoms such as bronchial asthma, allergic rhinitis, urticaria, atopic dermatitis. MCNS relapses by inhalants and food allergens. Lagrue et al. (1975) have found that more than 50% of MCNS patients have a significantly elevated serum IgE level in the acute nephrotic stage of MCNS and a decreased IgE level in remission state. The role of IgE in MCNS is suggested to be as follows: First, IgE induces vasoactive mediators from mast cells and macrophage, which increases vascular permeability. Secondly, IgE induces platelets activating factors, which elaborate inflammatory substances and cationic proteins from platelets and these proteins alter the glomerular anionic sites, resulting in the albuminuria of the nephrotic state of MCNS. Recently interleukin-4 has been identified as an important cytokine in IgE production which responds by involvement in IgE gene rearrangement (Ishizaka et al., 1990). Recent study suggests that elevated T helper cells (CD4+ cell) associated cytokines from these cells might play an important role in the pathogenesis (Tomizawa et al., 1985).

These results indicate the significance of interleukin-4 studies in MCNS. Interleukin-4 is also identified as a selective inducer of low affinity IgE receptors (Defrance, 1987). Two types of IgE receptors are identified: type I (high affinity receptor) and type II (low affinity receptor), of which only type II is induced by interleukin-4. This suggests that type II IgE receptors are closely linked to interleukin-4 activities. Type II IgE receptors in B-cells mediate the endocytosis process of the IgE-Ag complex, and also enhance the T-cell stimulatory effect of IgE-Ag. Soluble forms (sCD23) are shedded from the membrane of B-cells (mCD23). And it acts as a B-cell growth and B-cell differentiation factor and also enhances IgE half-life, after combining with IgE (Liu et al., 1991).

In conclusion elevated serum IgE levels, elevated membrane Fc ϵ RII and soluble Fc ϵ RII in MCNS patients indicate increased IL-4 activities in nephrotic state of MCNS. And these results also indicate activated T-cells, especially IL-4 secreting CD4+ T helper cells which are elevated in MCNS. Although considerable work remains to be done to define the pathophysiology and pathogenesis of MCNS, our study suggest that IL-4 and Fc ϵ RII may play an important role in the pathogenesis of MCNS.

ACKNOWLEDGEMENT

This study was supported by the Non directed Research Fund, Korea Research Foundation, 1990 and by a grant from the Korea Medical association (Dongshin-Smithklein Academy Award). A part of this paper was presented at the 23rd ASN (American Society of Nephrology) Meeting (1990). Washington, D.C. and at the 24th ANS Meeting (1991), Baltimore, U.S.A.

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