Dear Sir,

The production of the vascular permeability factor (VPF) from peripheral blood T lymphocytes in nephrotic patients has been confirmed by both Heslan et al. [1] and ourselves [2]. Furthermore, the appearance of significant proteinuria and reduction of anionic sites in the glomerular basement membrane of rat kidneys after infusion of the supernatant of T lymphocyte cultures from minimal change nephrotic syndrome (MCNS) has been demonstrated [3]. Nevertheless, the identity of the T lymphocyte subset which releases the VPF has remained obscure.

For the determination of the VPF released from T lymphocyte subsets, we investigated 8 nephrotic children in active and untreated stages. The peripheral heparinized venous blood was separated by Conray-Ficoll gradient. The mononuclear cells were filtered through a nylon wool column. The collected T cell-rich fractions were mixed with monoclonal anti-OKT4 or anti-OKT8 antibody, and incubated for 30 min at 4 °C. After the cells had been washed with PBS 3 times, they were incubated with 50 µl rabbit complement for 30 min at 37°C, and washed again with PBS 3 times. Cell viability was controlled with trypan blue exclusion dye. T cell subset content of the fractionated cell samples was checked by a cell sorter, after staining with monoclonal anti-OKT3, anti-OKT4 or anti-OKT8 antibodies. The percentage of CD3+ cells in the T cell-rich fraction was more than 90%, and that of CD4+ and CD8+ cells in T cell-rich fraction was 75 and 80%, respectively. Each cell sample was cultured in RPMI 1640 at a concentration of 1 × 10⁶/ml at 37°C and 5% CO₂ humidified atmosphere. After 48 h of culture, the supernatants were removed and stored at -80°C until use. The VPF assay on guinea pig skin was assessed according to Ovary’s method [4]. The diameter of the blue area surrounding each injected sites was measured and the area of blueing calculated. For statistic-
VPF production in the cultured unfractionated T cell-rich fraction and the CD4-rich fraction was significantly higher than in the cultured CD8-rich fraction (p < 0.01). MCNS has recently been reported to be a T lymphocyte-mediated disorder [5–8]. The VPF production from T lymphocytes in MCNS patients has also been confirmed [1–3]. In this paper, the prominent VPF production in the cultured CD4-rich fraction was demonstrated. However, the cause of such a VPF production of CD4⁺ cells in the T cell subsets is unclear. Anti-T cell antibodies appearing in the nephrotic stage of primary glomerulo-nephritis, which were detected by Nakabayashi et al. [9], might be associated with a decreased number of T 8 cells and enhanced function of T4 cells. Accordingly, it is presumed that the VPF production in MCNS may be related to CD4⁺ lymphocytes.

Further studies are needed to clarify the correlations between the VPF production of CD4⁺ lymphocytes and massive proteinuria in MCNS.

References


