Release of the Vascular Permeability Factor in Minimal Change Nephrotic Syndrome Is Related to CD4⁺ Lymphocytes

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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 1×10^6 /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 statisti-

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unfractionated CD4 rich CD8 rich T cell rich fraction fraction fraction

Fig. 1. VPF in T cell subset culture supernatants from patients with active MCNS. Mean $\pm$ SE, 1×10^6 cells in 1 ml of RPMI 1640 with 10% FCS incubated for 48 h. 0.1 ml of supernatant in each fraction was used for VPF assay.

cal analysis, the nonparametric Wilcoxon's rank sum test was used.

The blueing area induced by the supernatant from the CD8-rich fraction was less than 64.0 mm^2 . The mean blueing area was 41.6 mm^2 . However, the supernatant of CD4-rich fraction elicited a more prominent reaction, with a mean blueing area of 75.1 mm^2 . The supernatant of the unfractionated T cell-rich fraction gave a marked reaction as well, with a mean area of 87.8 mm^2 (fig. 1). The

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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.

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