The Vascular Permeability Factor Is a T Lymphocyte Product

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Dear Sir,

The vascular permeability factor (VPF) is a vascular-permeability-enhancing lymphokine produced in excess by concanavalin A (Con-A)-stimulated lymphocytes from patients with nephrotic syndrome [5, 10]. The identity of the cell subset implicated in VPF production is still being debated: while Shimizu [9] has reported that VPF is produced by B lymphocytes, for Tomizawa et al. [11] this lymphokine is produced by T cells.

In order to determine the VPF-releasing subsets we studied 10 adult patients (5 females and 5 males, 20–56 years old) with untreated nephrotic syndrome (protein-uria > 5 g/d) without renal failure. Histological findings included minimal change disease (3 cases), focal and segmental glomerulosclerosis (1 case), membranous glomerulonephritis (2 cases), type I membranoproliferative glomerulonephritis (4 cases). We filtered unfractionated blood lymphocytes through a glass-wool column and cultured them in the presence of Con-A as previously described [5]. To isolate lymphocyte subpopulations, venous blood samples were submitted to Ficoll-Hypaque gradient. Collected mononuclear cells were allowed to form E-rosettes with sheep red blood cells. A second gradient centrifugation was performed with these cellular complexes, and finally the cells were recovered, washed and treated with 0.17 M ammonium chloride to lyse attached red cells. Cell viability was controlled with trypan blue exclusion dye. T cell content of all fractionated cell samples was checked by staining with a monoclonal antipan-T-cell antibody (OKT3, Orthoclone) and UV microscopy. Each cell sample was cultured in RPMI 1640 at a concentration of 1×10^6 cells in 1 ml of medium at 37 °C and 5% CO₂. After 48 h culture, the supernatants were removed. Four different cultures were made: Culture A: unstimulated glass-wool separated lymphocytes; culture B: Con-A-stimulated glass-wool filtered lymphocytes; culture C: Con-A-stimulated E-rosette-depleted cells; culture D: Con-A-stimulated E-rosette-enriched T cells.

In these four cultures, the percentage of OKT3-positive cells differed. In cultures A and B, these percentages reached 60–70%. In culture C this percentage fell to below 20% whereas in culture D it rose to over 90% (table I).

We measured the VPF production on guinea-pig skin

Table I. Evan's blue spot diameter with VPF, supernatants of various lymphocyte subpopulations

Unstimulated lymphocytes
Con-A-stimulated lymphocytes
Con-A-stimulated depleted T cells
Con-A-stimulated enriched T cells

Wilcoxon signed-rank test signification: * $p < 0.01$ between A and B; ** $p = 0.02$ between B and C; *** < 0.02 between C and D; no difference between B and D.

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similar level in every case of nephrotic syndrome. This T cell release abnormality in nephrotic syndrome patients may be related to the T cell disorder recently suggested [6–8]. It may also be associated with a T cell cooperation defect as demonstrated by the decrease in IgG production in vitro [4].

In summary, we conclude that VPF is of T cell origin.

0

D

C

1/8 0

Fig. 1. VPF production by various lymphocyte subsets. A = Controls (unstimulated lymphocytes); B = Con-A-stimulated lymphocytes; C = Con-A-stimulated depleted T cells; D = Con-A-stimulated enriched T cells.

according to Ovary's method and we expressed the results as the average Evans' blue spot diameter in at least 2 animals; the positive threshold was previously defined at 6 mm. The nonparametric Wilcoxon signed rank test was used for statistical analysis.

VPF production in cultures A and B was significantly different ($p < 0.01$) with 9 out of 10 values higher in culture B than in culture A (fig. 1). Moreover, the comparison between cultures B and C also showed a significant difference ($p = 0.02$). Only 1 out of 10 samples was above 6 mm in culture C. Therefore, culture D yielded higher values and was significantly different from culture C for the VPF production ($p < 0.02$). Lastly the data in culture D were similar to those of culture B.

Our results demonstrate the predominant role of T lymphocytes in VPF production: in culture D with 90% of T lymphocytes we have the same higher VPF production that we obtained with glass-wool-filtered lymphocytes (culture B). On the other hand when less than 20% of T lymphocytes remains in culture C, we observe a significantly lower VPF production than in culture B; thus the removal of 70% of T lymphocytes decreases the VPF production by about 66%. T lymphocytes are then required to produce a significant amount of VPF.

Moreover, B lymphocytes and monocytes are not directly involved in VPF production, since VPF activity is low, in spite of their high concentration in culture C.

It remains difficult to determine whether the Con-A-stimulated T lymphocytes directly produce VPF or release an interleukine that can stimulate other cells to produce VPF.

Early data have demonstrated that VPF production occurs in various glomerular diseases [1–3,6]. Indeed we have observed a VPF production by T lymphocytes at a

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