Acta virol. **35**: 298-301, 1991

FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTIC SUBSETS IN HAEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS INFECTION

B. TURKOVIĆ[1], I. SVOBODA-BEUSAN[2]

[1]Institute of Public Health of Republic Croatia, Virology Division, 41 000 Zagreb; and [2]Institute of Immunology, 41 000 Zagreb, Yugoslavia

Received February 7, 1990

Summary. - Percentage counts of T-lymphocytes (helper and suppressor subpopulation) and B-lymphocytes were performed in the peripheral blood of two confirmed cases of HFRS infection using Coulter Clone monoclonal antibodies and Becton Dickinson flow cytometry. The individual values were compared with those from healthy subjects. HFRS patients had lower total T-cell (CD2) and T-helper (CD4 subset) counts. This seems linked with the role of the CD4 determinant as viral receptor. The measuring of specific lymphocyte helper and suppressor subpopulations might be useful in predicting the course of the disease and in following the effects of therapy.

Key words: haemorrhagic fever with renal syndrome (HFRS); Hantaan virus (HV); T-lymphocyte; flow cytometry

Decrease in the T_h-(helper) and increase in the T_s- (cytotoxic suppressor) subsets have been reported in some virus infections (Dauriac *et al.*, 1985; Stites *et al.*, 1986). Decreases in the numbers of T_h-lymphocytes have been in general reported with viruses that enter their T_h-lymphocytes as host cells by membrane fusion (Mims, 1986; Nicholson, 1989). Rise in the T_{sc} lymphocyte population, which is noticeable in a number of viral diseases (Schooley *et al.*, 1983; Melbye *et al.*, 1986; Sissons *et al.*, 1986) indicates that such disorder could be a frequent phenomenon. In HIV-infected patients a prognostic indicator is the number of $CD4^+$ cells in peripheral blood (Melbye *et al.*, 1986). If it is below 400 per mm^3 and if its decline is likely to continue, the diagnosis of AIDS is relevant. Consequently, there is probably every reason for employing CD4 cells for immunophenotyping of HIV infections. Very little is known, however, of the role of CD4 cells in HFRS infections.

The use of monoclonal antibodies (MoAb) with a specificity for lymphocyte subsets might provide a new and powerful tool for characterizing the immunologic status of virus-infected cases. The objective of the present investigation was to determine the immunologic status of the two cases of HFRS and esta-

blish which of the lymphocytic subsets were most affected by Hantaan virus (HV) infection.

The HV-76-118 and Fojnica antigens (Fojnica 2508, isolated in Yugoslavia) were propagated as described (Lee *et al.*, 1978). The antigens for the indirect immunofluorescence test (IF) were prepared on a Vero-E6 cells and used for IF staining as described (Lee, 1982). To be considered positive, specific IgM or IgG antibody titre to any of antigens tested had to be higher than 1:16. Lymphocytic population count was taken using fresh heparinized blood samples under conditions set by the Coulter Clone procedure (Coulter Clone, 1984). To exclude the influence of circadian rhythm, the samples were collected between 8 and 10 a.m. For isolation of lymphocytes use was made of the Ficol-Hypaque technique; the cells were stained with appropriate MoAb and analysed by FACScan flow cytometer (Becton Dickinson series). For IF staining lymphocytes were incubated with one of four mouse-antihuman MoAb (Coulter Immunology, Hialeah FL): T11 (CD2-pan T cells), T4 (CD4-T_h cells) T8 (CDS-T_{sc} cells), and B1 (CD20-pan B cells); and then with fluorescein-conjugated goat anti-mouse $(Fab)_2$ immunoglobulin. In each sample, 5000 cells were examined. Forward angle light scatter was used to discriminate between lymphocytes, monocytes, and debris. The mononuclear cell fraction was electronically gated to remove debris, dead cells, platelets, remaining erythrocytes and granulocytes. In both cases test samples gave a clearly discriminated region between positive and negative fluorescence. The fluorescence gain was adjusted to put this region in a standard channel before counting. Positive counts were corrected for background by subtacting control counts above this channel at the same gain setting.

In both HFRS infection suspects, antibody titres were confirmed by the IF test. Whereas sera from the patients with clinical signs of the disease revealed virus-specific IgG antibodies, they showed no IgM antibodies. Patient 1 had a higher (1:256) IgG antibody titre than patient 2 (1:16).

Lymphocyte subsets were identified phenotypically with MoAb by means of surface markers on T- and B-lymphocytes. Fig. 1 shows the results of fluorescence intensity testing for total T (CD2), T_h (CD4), and T_s (CD8) populations. T-lymphocyte histograms are shown for patient 1 and control blood with a marked reduction being evident in the relative proportions of T11 (CD2) and T4 (CD4) lymphocytes. The values of the CD8 subpopulation were not changed in relation to the normal value examined. B-lymphocytes were not changed either (data not shown). In practice, lymphocyte subpopulation values are often expressed as a CD4/CD8 lymphocyte ratio (I). This index is normally in the 1.8 - 2.2 range, because healthy subjects possess twice as many T_h as T_{sc} cells. In HFRS patients T_h and T_{sc} ratios were 0.75 (24/34) in case 1 and 1.12 (29/21) in case 2, respectively. In controls the I ratio values were found to be 2.06±0.59.

The functions of $CD4^+$ cells are diverse. They consist of cells that provide major support in B-cells response (helper-effector cells) and in those that induce suppressor cells (suppressor-inducer cells). The Coulter Clone T4 MoAb react with $CD4^+$ cells of both functions, i.e. helper-effector, and suppressor-inducer as well. Thus, in HFRS infection there appears to be a decrease in both $CD4^+$ cell subsets, though this was not established in a selective manner. In our cases the change in CD8 cells was uneven. Namely, a rise in their numbers occurred in patient 1 which may not have been entirely due to HV infection. Conversely, a small decrease in the CD8 subpopulation was

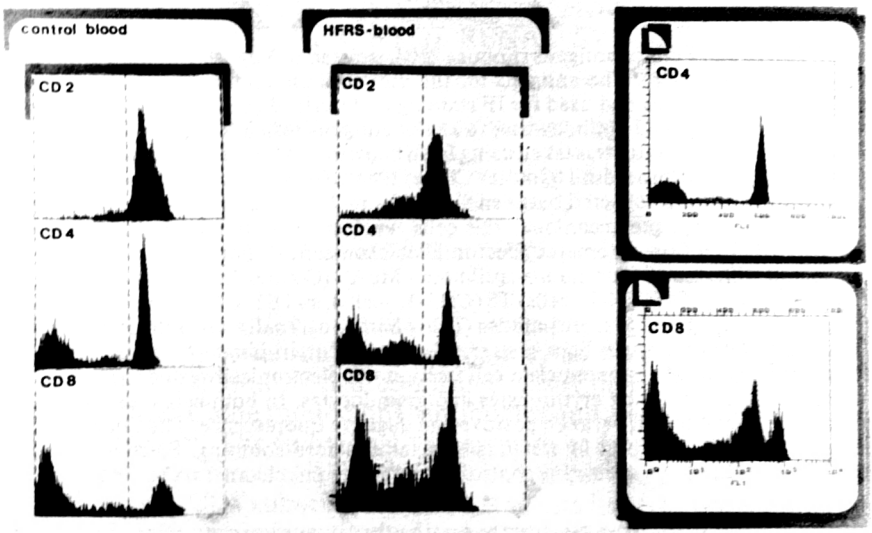

Fig. 1
Phenotypic analysis of T-lymphocytes
The cells were marked with the T11 (CD2), T4 (CD4), and T8 (CD8) MoAb. Comparison lymphocytes from the HV-infected patient, and from normal blood sample shows reduction in the CD2 and CD4 populations (arrows). The CD8 subpopulation values remained unchanged.

observed in patient 2. Incidentally, the CD8 cell increase must also have resulted from the infection by other virus, especially human immunodeficiency virus (HIV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV). The roles which these and, perhaps, other viruses in virus infection cases may play in contributing to the rise of CD8 cell population among virus-infected cases are unknown. Likewise, it would be of interest to determine the role of cytotoxic subpopulation in CD8 cells. Another open question is whether these cells act cytotoxically against virus-infected target cells or give any protection against virus infection. Since this investigation covered only two confirmed samples of HFRS, it permits no conclusions. Compared to healthy controls both cases had a decreased percentage of CD2 and CD4 cells.

The development of MoAb and flow cytometric technologies have enabled the identification of cells expressing new markers (phenotypes) ad a linking phenotypes with a differentiation between state and function. Comprehensive two-color immunofluorescence panel for virus infection, capable of including $CD2^+$ plus $CD20^+$ and $CD4^+$ plus $CD8^+$, as well as other subclass combinations have opened up new possibilities. With advances in clinical laboratory technology will be expanding as we explore more applications.

Acknowledgement. The authors would like to thank Mr. Wilim Crlenjak, BA, Institute of Public Health of SR Croatia, for the valuable help of translating the manuscript.

References

Coulter Clone (1984): Monoclonal antibodies. Coulter procedures. *Coulter Immunology,* Hialeah, Florida, U.S.A.

Dauriac, C., Guyotat, D., Vu Han, H., Magaud, J. P., and Fière, D. (1985): Analysis of early T4/T8 ratio following allogenic bone marrow transplantation: Correlation with acute Cytomegalovirus infection and acute graft-versus-host-disease. *Exp. Hematol.* **(S 17) 13,** 99.

Lee, H. W. (1982): Korean haemorrhagic fever. *Progr. med. Virol.* **28,** 96-113.

Lee, H. W., Lee, P. W., and Johnson, K. M. (1978): Isolation of the aetiologic agent of Korean haemorrhagic fever. *J. infect. Dis.* **137,** 298-308.

Mims, C. A. (1986): Interactions of viruses with the immune system. *Rev. clin. exp. Immunol.* **66,** 1-16.

Melbye, M., Biggar, R. J., and Ebbesen, P. *et al.* (1986): Long term seropositivity for human T-lymphotropic virus type III in homosexual men without the acquired immunodeficiency syndrome: Development of immunologic and clinic abnormalities. *Ann. int. Med.* **104,** 496-500.

Nicholson, J.K.A. (1989): Use of flow cytometry in the evaluation and diagnosis of primary and secondary immunodeficiency diseases. *Arch. Pathol. Lab. Med.* **113,** 598-605.

Schooley, R. T., Hirsch, M. S., Colvin, R. B., Cosimi, A. B., Tolkoff-Rubin, N. E., Mc Cluskey, R. T., Burton, R. C., Russell, P. S., Herrin, J. T., Delmonico, F. L., Giorgi, J. V., Henle, W., and Rubin, R. H. (1983): Association of herpesvirus infections with T-lymphocyte-subset alterations, glomerulopathy and opportunistic infections after renal transplantation. *N. Engl. J. Med.* **308,** 307-313.

Sissons, J.G.P., Borysiewicz, L. K., Rodgers, B., and Scott, B. (1986): Cytomegalovirus - its cellular immunology and biology. *Immunol. Today* **7,** 57-61.

Stites, D. P., Casavant, C. H., McHugh, T. M., Mods, A. R., Beal, S. L., Ziegler, J. L., Saunders, A. M., and Warner, N. L. (1986): Flow cytometric analysis of lymphocyte phenotypes in AIDS using monoclonal antibodies and simultaneous dual immunofluorescence. *Clin. Immunol. Immunopathol.* **38,** 161-167.