Scripta Scientifica Medica, vol. 30 (1998), pp. 131-136
Copyright © Medical University, Varna

FLOWCYTOMETRIC STUDIES OF CELL IMMUNITY IN PATIENTS WITH URAEMIA ON PERIODIC HAEMODIALYSIS AND AFTER RENAL TRANSPLANTATION

S. Staikova, S. Balev*, K. Nenov, K. Kisyova*, D. Nenov

*Department of Nephrology, Haemodialysis and Haematology and * Department of Pulmology, Clinical Immunology, Physiotherapy and Occupational Diseases, Medical University of Varna, Varna*

The course of a series of kidney diseases is characterized by impaired immunity additionally altered during haemodialysis and after renal transplantation. The study covered 50 patients divided into three groups: 16 patients with chronic renal failure (CRF), 23 ones on periodic haemodialysis (PHD), and 11 after renal transplantation. Flowcytometric immunophenotyping by means of FACS-analysis using monoclonal antibodies was carried out. The investigation aimed at revealing the changes in the cell immunity, activation of surface markers and adhesion molecules in these patients. Statistically significant changes in the expression of ICAM-1 adhesion molecules were established as followed: CD54+/CD4+ = 24,8 ± 11,6 (in CRF) against 14,9 ± 8,1 (in PHD) at $p < 0,05$ and CD54+/CD8+ = 17 ± 6,9 (in CRF) against 10,3 ± 4,8 (in PHD) at $p < 0,05$. A conclusion was drawn that immune response damage in PHD patients resulted mainly from the lowered expression of the adhesion molecules of the ICAM-1 immunoglobulin gene superfamily.

Key-words: Chronic renal failure, haemodialysis, flow cytometry, immunophenotyping, ICAM-1 adhesion molecules

There exists a serious disturbance in the reactivity of the patients with chronic renal failure (CRF) and on periodic haemodialysis (PHD) that is explained by their reduced resistance towards infection (2). Recently, Japanese investigators (4) reported a considerable damage of gamma-delta T-cells in patients on PHD but a less expressed one - in CAPD as compared with healthy controls. In these authors' opinion, the aforementioned cells were susceptible to activation-induced cell death (apoptosis). It was supposed that cell immunity could be influenced in PHD patients depending on the kind of dialyzer membrane and its biological compatibility.

Address for correspondence:
S. Staikova, Dept. of Nephrology, Haemodialysis and Haematology, Medical University, 55 Marin Drinov St, BG-9002 Varna, BULGARIA
E-mail: nephro@asclep.muvar.acad.bg

It is well known that there are lesions of the lymphocytic and polymorphonuclear leukocytic functions in patients on PHD. The process of extracorporeal circulation itself is accompanied by a periodic contact of blood with various artificial non-physiological materials such as dialyzer membranes, haemolines, puncture needles, catheters, etc. Depending on the composition of these materials and consumer means a change in the activity of mononuclear cells occurs resulting in a chain activation of whole cascades of cytokines, interleukins and other biologically active mediators as well (3).

The purpose of the present investigation is to compare the changes in the cell-mediated immunity in patients with CRF on PHD and after renal transplantation by means of studying the lymphocytic populations and subpopulations, some activation markers and adhesion molecules as well.

MATERIAL AND METHODS

The trial covered 50 patients (36 males and 14 females) aged between 25 and 60 years and hospitalized in the Clinic of Nephrology and Haemodialysis, Department of Nephrology, Haemodialysis and Haematology, Medical University of Varna. They were divided into three main groups: 1) 16 patients with CRF; 2) 23 patients on PHD with duration of dialysis treatment between one month and 12 years. All the PHD patients underwent 12-hour long haemodialysis procedures threefold weekly and were regularly given erythropoietin. Cellulose-acetate and polysulphonic dialyzer membranes were used, and 3) 11 patients (9 males and 2 females) aged between 40 and 60 years after renal transplantation predominantly from live donors. All the transplanted patients were administered corticosteroids and imuran but 8 of them only - additionally cyclosporin A. Immunophenotyping was performed according to the following protocol:

Samples: whole blood taken by venipuncture into K_3 heparin VACUTAINER®. Preparation of lymphocytes (Ly): standard procedure for two-colour direct immunofluorescence of Ly subsets in erythrocyte-lysed whole blood; washing (Washing solution), lysing (Lysing solution), and fixing with 1,5 % paraformaldehyde (CellFix). Monoclonal antibodies (MCA): combination of MCA conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE).

1) Cell immunity - Becton Dickinson (BD) Simultest IMKPlus reagent kit was used as followed: CD3/CD19 - total T Ly (CD3+) and total B Ly (CD19+); CD4/CD18 - T helper/inducer (CD4+) and T suppressor/cytotoxic (CD8); CD3/CD16+56+ - NK-natural killer (CD3-/CD16+56+).

2) Activated T Ly: a) early activated Th and Ts - by expression of

interleukin-2R (IL-2R) (CD25): CD25/CD4 - activated Th Ly; CD25/CD8 - activated Ts Ly; b) late activated total T Ly - by expression of CD3/HLA-DR.

3) Adhesion molecules: ICAM-1 (CD54), LFA-1 (CD11a) and MAC-1 (CD11b) - by expression on T Ly: CD54/CD4 - expression on Th and CD54/CD8 - for expression on Ts; CD11a/CD8 - expression on Ts and CD11b/CD8 - for expression on Ts.

For the cytometric analysis of Ly subsets, a FACSort flowcytometer (Becton Dickinson) was used, isotope control was done with LeucoGATE and Simulset IMK plus software was applied for data evaluation. A statistical data processing was performed after the method of Student-Fisher.

RESULTS AND DISCUSSION

As shown on Fig. 1, the changes of CD3 parameter consisted in an insignificant increase of total T Ly in the patients on PHD as compared to these with CRF but in a statistically significant increase of these lymphocytes in the transplanted patients ($p < 0,05$). The late activated total T Ly (CD3/HLA-DR) were considerably higher in the transplanted patients (Fig. 2). During the T Ly activation a cascade of intracellular biochemical processes was initiated accompanied by typical morphological alterations in blast transformation. It was obvious (Fig. 3) that B Ly (CD19) gradually reduced in the three patients' groups. This parameter was

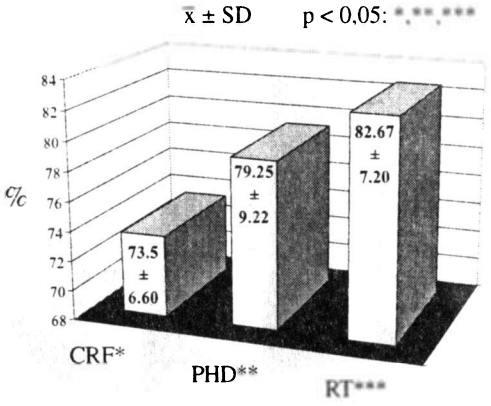

Fig. 1. CD3 in patients with CRF, on PHD, and after RT

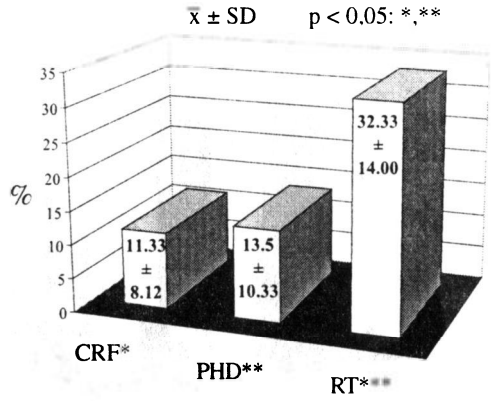

Fig. 2. Activated T-ly in patients with CRF, on PHD, and after RT

statistically significantly diminished in the patients after renal transplantation which testified to the effective immunosuppressive therapy. These results cound be also interpreted as a manifestation of the acute modulation of the phagocytic function by the extracorporeal circulation in the patients on PHD. Antibody deficiency is, in general, characterized by common bacterial infections (5).

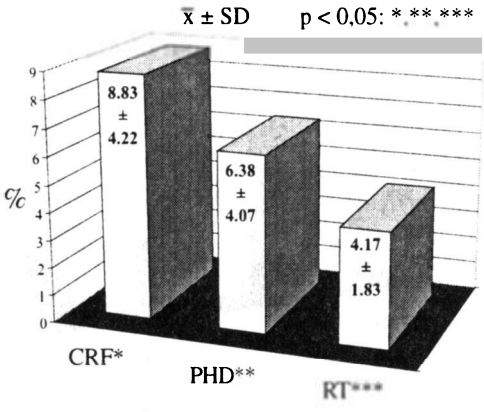

Fig. 3. CD19 in patients with CRF, on PHD, and after RT

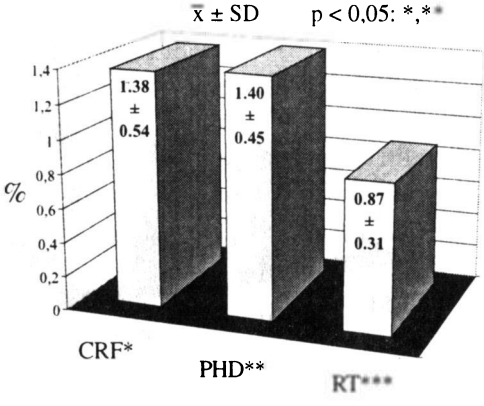

Fig. 4. CD4/CD8 in patients with CRF, on PHD, and after RT

The CD4/CD3 ratio (Fig. 4) reflects the processes of T-cell activation. It is considered as lowered when it is below 1,5 (3). The kidney transplantation statistically significantly reduces the CD4/CD8 ratio when compared with PHD alone. It has been established that when the CD4/CD8 index in the peripheral blood is higher then the perivascular ratio is higher, too, and thus the rejecting reaction is reversible (1).

It is known that ICAM-1 mediate the adhesion of T-cells with antigen-presenting cells towards the activated endothelium. ICAM-1 play the role of co-stimulating signals for T-Ly activation and realize the interactions between T- and B-Ly and the penetration of T-Ly into the extravasal space thus enabling the transendothelial migration (3). In our material, there were statistically significant differences in the expression of ICAM-1 adhesion molecules (Table 1). In the patients on PHD, the expression of the adhesion molecules on T-helper and T-suppressor cells is lower than that in the patients with CRF: CD54+/CD4+ = 24,8 ± 11,6 (in CRF) against 14,9 ± 8,1 (in PHD) at $p < 0,05$ and CD54+/CD8+ = 17 ±

Table 1

Expression of adhesion molecules on lymphocytes

MCA	CD54+CD3-	CD54+CD3+	CD54+CD4-	CD54+CD4+	CD54+CD8+	CD54+CD8-
PHD	14,8	20,5	33,6	14,9	33,4	10,3
SD	4	12,5	6,6	8,1	9,6	4,8
CRF	20,4	32,1	44,8	24,8	50,8	17
SD	5,7	17,8	9,4	11,6	13,7	6,9
p<	0,07	0,1	0,06	0,05	0,05	0,05

6,9 (in CRF) against 10,3 ± 4,8 (in PHD) at $p < 0,05$.

CONCLUSION

The changes in the lymphocytic populations and subpopulations such as CD3, CD3/HLA-DR, CD19, and CD4/CD8 as well as the decreased expression of the adhesion molecules of the ICAM-1 immunoglobulin gene superfamily in CRF patients as compared with that in the patients on PHD and after renal transplantation could be due to the disturbed immunologic homeostasis caused by several factors of the renal pathology typical of these patients such as infections, etiology of the basic disease, duration of PHD treatment, composition of the dialyzer membrane, kind of dialysis solution, erythropoietin treatment, etc. The action of these factors results in the decreased liberation of IL-2 and other mediators of inflammation such as leukotriens, TNF-alpha, IL-1, etc. (5). That is why each parameter should be individually interpreted taking into consideration the involvement of additional factors with the single patient.

REFERENCES

1. Наумова, Е. В: Клинична имунология. Под ред. Б. Божков, М. Огнянов. София, Знание, 1997, 393-400.- 2. Огнянов, М., И. Алтънкова. В: Имунология на вътрешните болести. София, Медицина и физкултура, 1990, 9-44.- 3. Cibrik, D. M., J. R. Sedor. In: Primer on Kidney Diseases. 2. ed. A. Greenberg, ed. San Diego, etc., Academic Press, 1998, 141-149.- 4. Matsumoto, J., T. Shinzato, I. Takai, et al. *Nephrol., Dial. Transplant.*, **13**, 1998, No 11, 2861-2866.- 5. Vanholder, R., R. De Smet, V. Jacobs, et al. *Nephrol., Dial. Transplant.*, **9**, 1994, No 9, 1271-1278.

Флоуцитометрични проучвания на клетъчния имунитет при болни с уремия, на периодична хемодиализа и след бъбречна трансплантация

С. Стайкова, С. Балев*, К. Ненов, К. Кисьова*, Д. Ненов

*Катедра по нефрология, хемодиализа и хематология и *Катедра по пневмология, клинична имунология, физиотерапия и професионални болести, Медицински университет-Варна*

Резюме: Редица бъбречни заболявания протичат с увреден имунитет, който допълнително се променя при хемодиализа в зависимост от вида на прилаганите мембрани. Изследвани са 50 болни, разпределени в 3 групи: 16 - с хронична бъбречна

недостатъчност (CRF), 23 - на периодична хемодиализа (PHD) и 11 - след бъбречна трансплантация. Проведено е флоуцитометрично имунофеногипизиране чрез FACS-анализа с използване на моноклонални антитела. Целта на изследването е установяване на промените в клетъчния имунитет, както и на активирането на повърхностните маркери и адхезионните молекули у болните. Намерени са статистически значими промени в експресията на адхезионните молекули ICAM-1: CD54+/CD4+ = 25 ± 10,2 (при CRF) срещу 15 ± 7,1 (при PHD) ($p < 0,05$) и CD54+/CD8+ = 17 ± 6,6 (при CRF) срещу 10 ± 4,5 (при PHD) ($p < 0,05$). Авторите заключават, че увреждането на имунния отговор се увеличава у диализно болните главно в резултат на снижената експресия на адхезионните молекули на имуноглобулиновата генна суперфамилия ICAM-1.