

Effect of Haemo- and Peritoneal Dialysis on the Cell-Mediated Immune Response in Chronic Uraemia*

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Lymphocytopenia, decreased spontaneous rosette formation, and a decreased T lymphocyte count have been found in patients with non-uraemic glomerulonephritis (71 cases) and in different stages of uraemia (68 cases). In chronic glomerulonephritis and in the early stage of uraemia, cell-mediated hypersensitivity (lymphocyte migration inhibition) to glomerular basement membrane (GBM) characteristic of glomerulonephritis could be demonstrated. Hypersensitivity disappeared in the terminal stage of uraemia indicating endogenous immunosuppression.

Haemodialysis (HD) and peritoneal dialysis (PD) failed to influence the leukocyte count, spontaneous rosette formation, and T lymphocyte count, but caused the reappearance of cell-mediated hypersensitivity to GBM antigen.

Thus uraemia is not responsible for T lymphocytopenia, and the immunosuppression observed in uraemia is independent of the decreased T cell count. On the other hand, the suppression of specific cell-mediated reactivity is the result of the uraemic state, probably by way of toxic metabolite(s). The factor(s) responsible for immunosuppression is (are) dialysable.

The cell-mediated immune response in uraemia has been extensively studied, but the data available are controversial. Thymic atrophy [34], lymphocytopenia [3, 9, 17, 24, 30, 34] and T lymphocytopenia [9, 24, 26] found in chronic uraemia point to immunosuppression. However, some authors maintain that neither lymphocytopenia [13] nor T lymphocytopenia [13, 16] can be demonstrated in uraemia. The data referring to the *in vitro* survival of lymphocytes are also contradictory: it may be reduced [1, 5], but may also be normal [22]. The response to mitogens may be reduced [10, 13, 16, 23, 28, 29], normal [1, 8, 12, 27], or increased [3, 4]. Daniels et al. [5] reported on increased spontaneous blastogenesis of lymphocytes in uraemic patients, which made the evaluation of the results difficult. The lymphocytes in mixed lymphocyte culture may be hyporeactive [6, 10] or normoreactive [27, 28]. Reduced cell-mediated reaction has also been reported in response to stimulation with specific antigens [1, 7]. According to some authors, the plasma of uraemic patients does not interfere with the response to mitogenic agents of lymphocytes of healthy subjects [10], while others maintain that it has

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a suppressive effect [20, 22, 28, 30]. Delayed cutaneous reaction to various antigens may show anergy [5, 10, 14, 27, 34], but may also be normal [10, 13]. The survival of skin transplants is prolonged in uraemia [2, 18, 31].

The data concerning the effect of HD on cell-mediated immunity are not in agreement either. According to some authors there may be a decrease in immunosuppression [10, 17], while others have found it unchanged after HD [1, 8, 13, 20, 27, 28]. It has been suggested that the factor(s) responsible for immunosuppression is (are) dialysable [22], but the opposite has also been stated [12, 13, 20, 28].

No studies have so far been performed concerning the effect of PD on cell-mediated immune response.

In the present study of uraemia in chronic glomerulonephritis an answer has been sought to the following questions:

1. Can lymphocytopenia, decreased rosette formation and T lymphocytopenia be found in uraemia?
2. Is the specific anti-GBM cell-mediated response, frequently observed in chronic glomerulonephritis as a sure sign of immune activity, also demonstrable in different stages of uraemia?
3. What are the effects of HD and PD on the above parameters?

Material and methods

Two groups of patients were investigated. In Group I there were 71 non-uraemic chronic glomerulonephritic patients (serum creatinine 2 mg/100 ml), while Group II comprised 68 uraemic patients. Three subgroups were distinguished within the uraemic group depending on the severity of uraemia: Group II/A (serum creatinine 2–4 mg/100 ml), Group II/B (serum creatinine 4–10 mg/100 ml), and Group II/C (serum creatinine 10 mg/100 ml). During the study period the patients received neither steroid nor cytostatic drugs. Twenty healthy subjects served as controls.

Leukocyte count, total lymphocyte count

The leukocyte count (mean \pm SE = $6000 \pm 300/\text{mm}^3$) and the total lymphocyte count (mean \pm SE = $2200 \pm 200/\text{mm}^3$) were established in the peripheral blood using the standard procedures.

Spontaneous rosette-forming activity

The percentage of lymphocytes forming rosettes with sheep red cells spontaneously was established by the slightly modified method of Jondal et al. [11]. From 10 ml heparinized venous blood the lymphocytes were isolated on Ficoll-Uromiro gradient. After repeated washings the cell count was stabilized at 1×10^6 lymphocytes/ml, and 0.1 ml of the lymphocyte suspension was incubated with

0.5 ml 5% sheep red cell suspension at 4°C for 1 h. The percentage of spontaneously rosetting lymphocytes was counted in a Bürker chamber. In the control group the mean \pm SE was $65 \pm 1\%$.

Total T cell count

From the total lymphocyte count and the percentage value of spontaneously rosetting lymphocytes the total T cell count was established. In the control group the mean \pm SE was $1400 \pm 110/\text{mm}^3$.

Preparation of GBM antigen

Glomeruli were isolated from fresh human cadaver kidney according to the method of Krakower and Greenspon [15], and solubilized according to Rocklin [25]. The solubilized GBM antigen was applied in $300 \mu\text{g}/\text{ml}$ concentration.

Leukocyte migration test

The method of Søbørg and Bendixen [32, 33] was modified for the study of leukocyte migration inhibition. 20 ml heparinized blood was mixed with a 5% dextrane solution and allowed to stand at 37°C for 1 h. The plasma rich in leukocytes was pipetted off, and the sediment was washed three times in Parker-199 medium after the contaminating red cells had been haemolysed. The leukocyte suspension (cell count $7 \times 10^7/\text{ml}$) was transferred into glass capillary tubes and centrifuged. The capillary tubes containing the cells were fixed in plastic chambers. Each chamber contained three tubes. The control chamber contained only medium, while the test chambers also soluble GBM antigen. Following incubation at 37°C for 20 h, the results were read by a planimetric method. The migration index (MI) was computed as follows:

$$\text{MI} = \frac{\text{mean area of migration in the presence of antigen}}{\text{mean area of migration in the absence of antigen}}$$

The mean MI \pm SE in the controls was 1.00 ± 0.02 , the normal range being 0.80–1.20. MI values below 0.80 indicated inhibition, while those higher than 1.20 signalized stimulation.

Haemodialysis

HD was performed using a Kiil-Lucas type apparatus with 150–200 ml/min blood flow rate and 500 ml/min fluid flow rate. The total period of dialysis was, depending on the condition of the patient and the residual renal function, 16–20 $\text{m}^2/\text{h}/\text{week}$. PT 150 cuprophane membrane was used.

Peritoneal dialysis

PD was performed by infusion technique through a Tenckhoff catheter. The mean duration of dialysis was 32–40 h/week, the amount of dialysis fluid, 50 l, and treatment was performed twice weekly. Peridisol 1 DK fluid (Human, Budapest) was used.

Two-sample *t*-test was used for statistical analysis of the results.

Results

In the group of non-uraemic glomerulonephritic patients (Group I) and in different phases of uraemia (Groups II/A, B, C) the leukocyte count was normal, whereas the number of lymphocytes decreased, spontaneous rosette formation was reduced, and the number of T cells decreased as compared with the controls. The difference was significant statistically (Fig. 1).

A significantly decreased MI was obtained in the presence of GBM antigen in the non-uraemic chronic glomerulonephritic patients (Group I), and in the first (Group II/A) and second (Group II/B) stages of uraemia. This sign was interpreted as indicating specific cell-mediated hypersensitivity to GBM antigen. In advanced uraemia (Group II/C), MI did not differ from the control value, indicating that endogenous immunosuppression had developed. Migration stimulation occurred in a very high percentage in Group II/C, unlike in Groups I, II/A and II/B (Fig. 2).

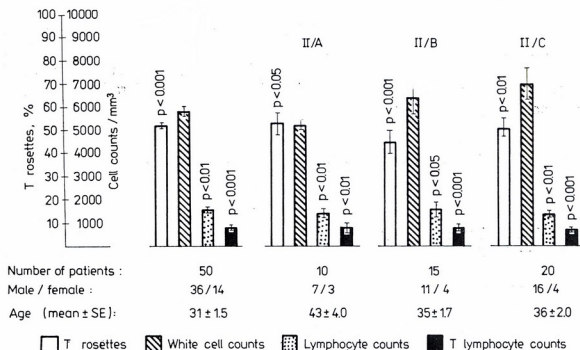


Fig. 1. Leukocyte count, lymphocyte count, spontaneous rosette formation and T lymphocyte count in chronic glomerulonephritis and in different phases of uraemia

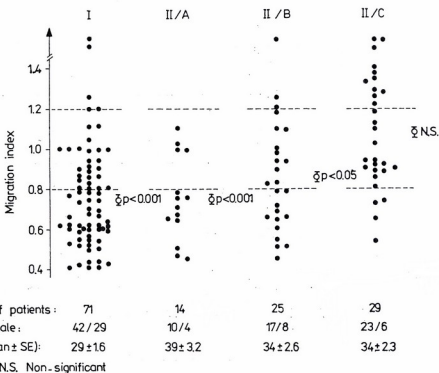
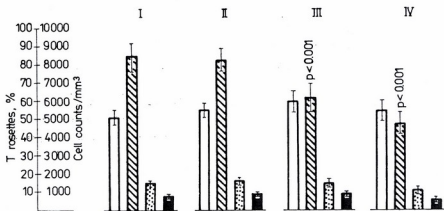
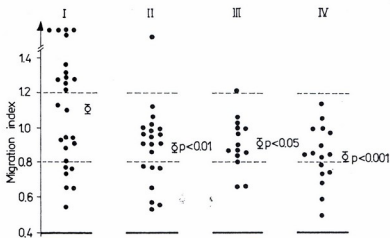


Fig. 2. MI in chronic glomerulonephritis and in different phases of uraemia



| Number of patients: | 15 | 13 | 10 | 10 |
|--|------------|----------------------------|------------------------------|------------------------------|
| Blood urea nitrogen, mg/100 ml (mean ± SE) | 130 ± 9.8 | 95 ± 9.7* | 71 ± 9.6* | 83 ± 6.8* |
| Serum creatinine, mg/100 ml (mean ± SE) | 12.6 ± 1.0 | 10.7 ± 1.1** | 10.5 ± 0.9** | 14 ± 1.5** |
| I Before haemodialysis | | II After 1-5 haemodialyses | III After 6-20 haemodialyses | IV After 21-40 haemodialyses |
| * Significant | | ** Non-significant | | |
| □ T rosettes | | ▨ White cell counts | ▩ Lymphocyte counts | ■ T lymphocyte counts |

Fig. 3. Effect of HD on leukocyte count, lymphocyte count, spontaneous rosette formation and T lymphocyte count



| | I | II | III | IV |
|--|------------------------|----------------------------|------------------------------|------------------------------|
| Number of patients: | 26 | 21 | 14 | 16 |
| Blood urea nitrogen, mg/100 ml (mean ± SE) | 118 ± 75 | 86 ± 72* | 79 ± 81* | 82 ± 5.0* |
| Serum creatinine, mg/100 ml (mean ± SE) | 11.8 ± 0.8 | 10.2 ± 0.8** | 10.6 ± 0.8** | 13.9 ± 0.9** |
| | I Before haemodialysis | II After 1-5 haemodialyses | III After 6-20 haemodialyses | IV After 21-40 haemodialyses |

* Significant ** Non-significant

Fig. 4. Effect of HD on MI

As a result of 5–20 periods of HD as well as of more than 20 periods, the leukocyte count decreased but the total lymphocyte count, spontaneous rosette formation, and T cell count remained unchanged as compared with the predialysis values (Fig. 3).

Even 1–5 periods of HD reduced the MI significantly as compared both with the predialysis value and with the controls. Migration stimulation was not observed after HD (Fig. 4).

Pd (mean total period 160 h, range 24–430 h) did not affect the leukocyte count, total lymphocyte count, spontaneous rosette formation and T cell count. At the same time, the MI decreased in 50% of the cases as compared with the predialysis value. The difference was significant statistically (Fig. 5).

Discussion

Uraemia is a long process, which should be subdivided into stages for the purpose of studying the patients' cell-mediated immune response. Our data have shown uraemia to be an immunosuppressive state, but only in its terminal stage.

Although the cell-mediated immune response has been extensively studied in uraemic patients, the results obtained, especially those reported recently, are contradictory. This may be attributed to several factors. Earlier the possibilities to treat uraemia were limited and unsatisfactory. The patient populations investigated

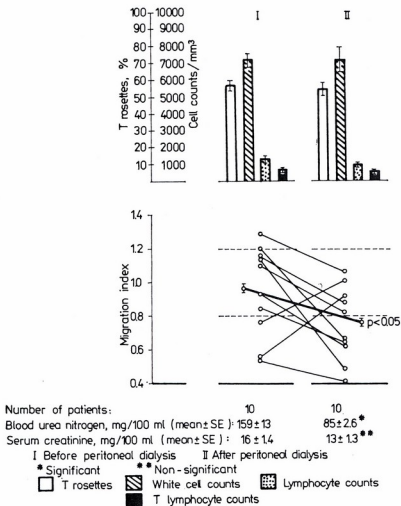


Fig. 5. Leukocyte count, lymphocyte count, spontaneous rosette formation, T lymphocyte count and MI before and after PD

by the authors of these early reports were inhomogeneous, comprising uraemias of widely varying origin. Also, the number of cases analysed was usually low and, what is more important, no stages had been distinguished within the course of uraemia. The situation has been further complicated by the fact that the role of cell-mediated immunity in the pathogenesis of nephropathies leading to uraemia has not been sufficiently clarified. Therefore, it is hard to say whether the deficient immune cell function is connected with the primary disease or with uraemia.

Our investigations have demonstrated lymphocytopenia, reduced spontaneous rosette formation, and T lymphocytopenia in chronic glomerulonephritis. The same findings were obtained in different stages of uraemia. Thus lymphocytopenia, reduced rosetting and decreased T cell count are not the result of uraemia, at least if it has developed in chronic glomerulonephritis. These changes thus cannot be attributed to uraemic toxicosis, and, consequently, the reduced cell-mediated

immunity observed in uraemia is not connected with the changes in these parameters.

Leukocyte migration test revealed specific cell-mediated hypersensitivity to GBM antigen in a great number of our non-uraemic glomerulonephritic cases. This, in our opinion, may play a role in the progression of the disease [21].

In the first and second stages of uraemia of chronic glomerulonephritic patients specific cell-mediated immunity to GBM antigen was also frequently found. This may suggest that also in uraemia — especially in its first two stages — there may be immunological activity, a point which ought to be considered when weighing the therapeutic possibilities.

In the terminal stage of uraemia, when HD or PD treatment must be applied, specific immune reactivity cannot be demonstrated indicating the development of endogenous immunosuppression. In this stage migration stimulation was found, which might be interpreted as a sign of cellular hyporeactivity and immunosuppression.

HD did not induce further depletion of lymphocytes and failed to affect the spontaneous rosette formation and T cell count. At the same time, it caused the reappearance of specific cell-mediated immunity. This also indicates that the uraemic T lymphocytopenia is not responsible for the cellular hypofunction, and that the plasma factor(s) of immunosuppression are dialysable.

PD did not affect the total lymphocyte count, rosetting activity and T cell count. It re-established the specific cell-mediated response indicating that the peritoneum is permeable to suppressor factor(s).

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