Diminished adhesion of CD4+ T cells from dialysis patients to extracellular matrix and its components fibronectin and laminin

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Abstract

Background. Cell-mediated immunity is impaired in uraemia. The recognition and ensuing interactions of immune cells, such as CD4+ T lymphocytes, with adhesive glycoproteins of the extra-cellular matrix (ECM) are mediated by integrins of the β1 subfamily. We have previously demonstrated that uraemic sera inhibit the proliferation and adhesion of normal CD4+ T cells to ECM components. In the present study, the adhesive capacity of CD4+ T lymphocytes of dialyzed patients (both haemodialysis [HD] and continuous ambulatory peritoneal dialysis [CAPD]) was evaluated.

Methods. Adhesion of CD4+ T cells from dialysis patients to intact ECM and its immobilized moieties, fibronectin (FN) and laminin (LN) was measured following phorbol-12-myristate-13-acetate (PMA) stimulation. In addition, cell surface expression of β1 integrins (VLA 4–6) was determined by FACScan analysis.

Results. Compared to normal cells, CD4+ T cells of dialysis patients demonstrated a significantly reduced adhesion to ECM, FN and LN (27–28 vs 52–55%, P < 0.001). This decreased adhesive capacity was not normalized upon incubation of the cells with normal sera. Cell surface expression of β1 integrins was not modified. The inhibition of cell adhesion was more pronounced in CAPD patients (23–24% vs 29–30% in HD, P < 0.02). Serum albumin correlated directly with cell adhesion. Aged HD patients’ T cells demonstrated increased adhesion to ECM and its ligands, whereas a reverse trend was demonstrated in the CAPD group.

Conclusions. T cells of dialysis patients exhibit abnormal adhesive activity, which may be due to an acquired cellular defect induced by uraemic milieu. CAPD patients show a greater degree of adhesion impairment, possibly due to their lower concentrations of serum albumin.

Key words: CD4+ T cell; cell adhesion molecules; dialysis; extracellular matrix; integrins; uraemia

Introduction

An immunodeficient state is a recognized feature of end-stage renal disease (ESRD) manifest by prolonged tolerance to allografts [1], increased susceptibility to infections [2,3], an abnormally high incidence of neoplasia [4], anergy and a defective response to vaccination [5,6]. The pathogenesis underlying this impaired immunity is multifactorial, and concerns, among other things, T cell function, although the ratio of CD4 and CD8 T cell subsets is within the normal range [7]. Uraemic serum markedly diminishes the proliferative response of normal T cells [8,9], suggesting the presence of circulating inhibitory substances. Uraemic T lymphocytes demonstrate an impaired blastogenic response to mitogens and antigens [10]. The defective proliferation of T cells is associated with a decreased production of interleukin-2 (IL-2) and interferon gamma (IFN-γ) [11].

The occurrence of an adequate immune response requires the migration of lymphocytes from blood vessels into inflammatory sites [12]. These lymphocytes come in contact with the extra-cellular matrix (ECM), which is composed of collagens, glycoproteins, proteoglycans, and associated molecules, such as fibronectin (FN) and laminin (LN) [13]. Apart from its mechanical role in the support and maintenance of tissue structure, the ECM, and its major ligands, also serve as modulators of cell activation, adhesion, growth, and differentiation [14]. The recognition, binding, and ensuing interactions of immune cells with the glycoproteins of the ECM are mediated by integrin receptors of the β1-subfamily, also designated very late antigen (VLA) proteins. Defects in cell-matrix adhesion may lead to immunodeficiency. Uraemic sera inhibit the adhesion of CD4+ T cells from normal subjects to ECM, FN, and LN and their subsequent proliferation.
[15]. In the present study, the adhesion of CD4+ T cells from dialysed patients, to ECM, FN, and LN was determined in vitro, in order to evaluate whether these cells also exhibit a reduced adhesion due to an intrinsic cellular defect.

Subjects and methods

Patients

The blood of 26 dialysis patients and 13 age and sex matched healthy volunteers (control group), was obtained by peripheral vein puncture. Of the 26 patients, 15 were on haemodialysis (HD) and 11 on continuous ambulatory peritoneal dialysis (CAPD). There were no patients with cachexia, diabetes mellitus, malignancies, recent infections, systemic disease (vasculitis, systemic lupus erythematosus, etc.), or those receiving immunosuppressive therapy. Haemodialysis was performed using either cuprophane or cellulose triacetate–polysulfone dialyzers (‘more biocompatible membranes’) with bicarbonate as buffer. The average Kt/V value achieved was 1.27. Blood samples were obtained just prior to a haemodialysis session. CAPD patients were dialyzed using Dialine® peritoneal dialysis solutions (Travenol, Ashdod, Israel), and the mean weekly urea Kt/V was 1.92. All patients were considered to be well dialysed during the 3 months prior to their participation in the study, as judged by clinical and kinetic modeling criteria. Eighteen patients were administered recombinant human erythropoietin in order to maintain serum haemoglobin >11g/dl. Five patients were able to maintain their serum haemoglobin >11g/dl without EPO usage. The remaining three patients had serum haemoglobin between 9–10 g/dl because EPO therapy was started only 1 month prior to their participation in the study.

Preparation of human CD4+ T cells

CD4+ T cells were purified from the peripheral blood mononuclear leukocytes of dialysis patients and healthy donors as previously described [16]. Briefly, the mononuclear cells were isolated on a Ficoll gradient, washed, and incubated (37°, 10% CO₂-humidified atmosphere) in tissue culture plates. After 2 h, the non-adherent cells were removed and enriched for T cells by passage through nylon wool columns (Polysciences, Inc. Warrington, PA). CD4+ T cells were negatively selected using a mixture of anti-CD8, CD19, and CD14 mAb conjugated to magnetic beads (Advanced Magnetics Inc., Cambridge, MA). The resulting cell population consisted of >90% CD4+ T cells.

Quantitation of CD4+ T cell adhesion

The adhesion of CD4+ T cells to intact ECM and immobilized FN and LN was assessed as previously described [16]. Briefly, freshly isolated bovine corneal endothelial cells (5 × 10⁴ cells/ml) were cultured in flat-bottom 96-well plates in DMEM supplemented with 5% dextran T-40, bovine calf serum, fibroblast growth factor (100 ng/ml), and antibiotics. After 6–8 days at 37°C in a 10% humidified atmosphere, the confluent layers of endothelial cells were dissolved by exposure to PBS/0.5% Triton 0.1%, 20 mM NH₄OH for 3 min at 22°C. This procedure yielded an intact ECM attached to the entire surface area of the wells which were free of serum proteins, nuclei, and cytoskeletal and cellular debris. To immobilize ECM ligands, FN or LN (1 mg/50 μl of PBS/well) were incubated (1 h, 37°C) in flat-bottomed microtitre plates. The plates were then washed with PBS and the remaining binding sites blocked (1 h, room temperature) with 2% BSA in PBS. Purified human CD4+ T cells (n=10⁴) were labeled (1 h, 37°, 10% CO₂) with Na¹⁹⁵ CrO₄ (New England Nuclear, Boston, MA; 3–5 μCi/10⁶ cells/100 μl of FCS). The cells were then washed three times with PBS, counted, and resuspended in adhesion medium (RPMI 1640 medium supplemented with 2% BSA, 1 mM Ca²⁺, 1 mM Mg²⁺, 1% sodium pyruvate, and 1% HEPES). [¹⁹⁵ Cr]-labeled T cells were seeded onto ECM-, FN- or LN-coated microtitre wells and incubated for 30 min at 4°C. Phorbol-12-myristate-13-acetate (PMA) (25 ng/ml, final concentration) was added to the wells to activate the cells. After an incubation (1 h, 37°C, 10% CO₂) the plates were washed with PBS to remove non-adherent cells, and the adherent cells were lysed with NaOH (1 N) or Triton 0.1%. The same procedures were performed with cells incubated overnight with sera from healthy donors. The amount (c.p.m.) of [¹⁹⁵ Cr] in the resulting supernatants corresponded to the percentage of bound cells. For each experimental group the results were expressed as the mean±SD per cent of T cells bound in triplicate wells. The percentage of bound cells per well was calculated as follows:

\[
\frac{a/b}{c/R} \times 100
\]

where:

- a = c.p.m. of residual cells
- b = spontaneous release of [¹⁹⁵ Cr] from the added cells
- c = total c.p.m. of cells added

Cell surface expression of integrins

Expression of integrins on the cell surface of resting and activated (following stimulation with PMA [25 ng/ml for 30 min, 37°C]) CD4+ T cells was determined by indirect immunofluorescence. Purified CD4+ T cells (10⁶/ml, final concentration) were preincubated (48 h, 37°C) with adhesion medium. The T cells were then washed and incubated (30 min, 4°C) with murine anti-human integrin mAbs (anti VLA-4, anti VLA-5, or anti VLA-6 diluted 1:400, in PBS containing 0.05% BSA and NaCl) or murine anti-human CD29 (common β₁ chain of the integrin) mAb (diluted 1:400). Next, the T cells were washed and incubated (30 min, 4°C) with FITC-conjugated Fab fragments of goat antimouse Abs (Jackson Immuno Research Laboratories, Inc., West Grove, PA). The fluorescently-labeled cells were then washed extensively, fixed with PBS containing 1% paraformaldehyde, and subjected to flow cytometric analysis in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Expression of integrins was then determined as the difference between cells stained with relevant anti-integrin antibodies and those stained with the isotype matched irrelevant control antibodies. The results were depicted as histograms, the ordinate indicating number of cells and the abscissa the intensity of fluorescence.

Control group CD4+ T cells were incubated with adhesion medium and with serum from dialysed patients.

Laboratory methods

Serum haemoglobin, creatinine, Ca, P and albumin were estimated by using standard laboratory procedures. PTH levels were determined by using N-tact® PTH SP Kit
Kt/V values in HD patients were calculated by the following formula:

\[ \text{Kt/V} = 1.18 \times (\ln R) \text{ (post/pre urea ratio)} \] [17], and in CAPD patients, Kt/V index was calculated according to the formula introduced by Gotch and Sargent [18].

Reagents

Human FN, LN, and BSA were obtained from Sigma Chemical Co. (St Louis, MO); monoclonal antibodies (mAb) to human VLA-4 (α4, clone P4G9) and 5 (α5, clone P1D6) were purchased from Telios Pharmaceuticals Inc. (San Diego, CA). mAb to human VLA-6 integrin (α6, CDW49 f GoH3) from CLB (Amsterdam, Holland); anti-CD29 mAb and anti-human CD3 mAb from Serotec (Oxford, UK). HEPES buffer and RPMI 1640 were purchased from GIBCO (Grand Island, NY). Foetal calf sera (FCS) and phosphate-buffered saline (PBS) were obtained from Biological Industries (Kibbutz Bet Haemek, Israel).

Statistical analysis

Differences between groups were analysed by the Student t-test. P values < 0.05 were considered to be statistically significant. To correlate adhesive capacity of the T cells with patients' age, duration of chronic renal failure or dialysis, Kt/V value, type of dialyzer, serum haemoglobin, creatinine, albumin, CaXP product, parathyroid hormone (PTH) levels, dose of erythropoietin (EPO), and the calculation of Pearson’s coefficient was performed.

Results

Patients

Demographic data of HD and CAPD patients are shown in Table 1. Mean age was 50.5 ± 17 years and 52.7 ± 19 years in HD and CAPD, respectively. There were 16 males (nine on CAPD), and 10 females (two on CAPD). HD patients had been dialyzed for almost 6 years (71.5 ± 71 months), and those on CAPD for 1.5 years (19.8 ± 22.2 months). Eight of the HD patients were treated with either cellulose triacetate or polysulfone, and seven used cuprophane dialyzers.

Table 1. Demographic data of HD and CAPD patients

<table>
<thead>
<tr>
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<tr>
<td>No:</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Sex (f/m):</td>
<td>8/7</td>
<td>2/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.5 ± 17 (27–75)</td>
<td>52.7 ± 19 (17–80)</td>
</tr>
<tr>
<td>Treatment duration (months)</td>
<td>71.52 ± 71.16 (6–276)</td>
<td>19.8 ± 22.2 (3–72)</td>
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<tr>
<td>Kt/V</td>
<td>1.27 ± 0.2 (1.03–1.66)</td>
<td>1.92 ± 0.27 (1.64 ± 2.5)</td>
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Date are shown as mean ± SEM.

Adhesion of T cells of dialysis patients to ECM, FN, and LN

Fifty-five per cent of T cells from healthy donors (control group) adhered to ECM, and 52% to immobilized FN and LN (Figure 1). In contrast, T cells of dialysis patients demonstrated a significantly lower adhesion capacity to ECM, FN, and LN (27, 27, and 28%, respectively, P < 0.001). Incubation of CD4+ T cells of dialysis patients with normal sera did not reverse their adhesion defect.

A significantly lower adherence to FN and LN was found in CD4+ T cells of CAPD patients compared to those treated by HD (24% and 23% vs 29% and 30%, respectively, P < 0.02)(Figure 2). Adherence to ECM (CAPD vs HD) was similarly decreased, although this did not attain statistical significance.

Fig. 1. T cell adhesion to fibronectin, laminin and extracellular matrix in all dialysis patients (group P). *P < 0.001 compared to results obtained from healthy donors (group C).

Fig. 2. T cell adhesion to fibronectin and laminin in haemodialysis (HD) vs CAPD (PD) patients. *P < 0.002 for fibronectin and laminin.
There were no significant correlations between the percentage of adherent cells and duration of chronic renal failure or dialysis, Kt/V value, type of dialyzer, serum haemoglobin, creatinine, Ca × P product, PTH levels, and the dose of EPO in patients with haemoglobin levels > 11 g/dl.

Significant correlations were found between:

(i) age and adherence to LN ($r = 0.51$, $P < 0.05$ and $r = -0.67$, $P < 0.03$ in HD and CAPD, respectively). A similar tendency regarding adherence to FN and ECM was noted, although this did not reach statistical significance.

(ii) Serum albumin level (of all the patients considered as a whole) and adherence to ECM, FN, and LN ($r = 0.51$, 0.51, 0.57, respectively, $P < 0.01$) (Figure 3). On separating HD from CAPD, the correlation to serum albumin was seen to be mainly based on the CAPD cohort whose albumin level was significantly lower than that of HD patients ($3.66 ± 0.10$ vs $4.22 ± 0.09$ g/dl, respectively, $P < 0.001$).

With regard to the other parameters known to affect the immune response in uraemia, such as serum haemoglobin, creatinine, Ca (including calculated ionized Ca), Ca × P product, and PTH levels, no differences were found between patient groups.

Cell surface expression of $\beta_1$ integrins on T cells

Because defective patients’ T cell adhesion to ECM, FN, and LN could be due to modified expression of surface $\beta_1$ integrin receptors, the level of $\beta_1$ integrins on the T cell surface was evaluated by FACSscan analysis. Surface expression of VLA integrins on T cells (resting or activated) was not affected as reflected by the number of positively stained cells and the density of integrin receptors on those cells.

Discussion

Defective T cell function, as shown by a depressed proliferative response to non specific stimuli, consti-
tutes an integral part of the immunodeficiency syndrome observed in ESRD [8,10]. This impaired function is due to the effect of circulating inhibitors in uraemic sera [8,9] and, possibly, to an acquired functional defect of T cells of dialysis patients. Uraemic sera have also been shown to inhibit the adhesive and proliferative capacity of normal T cells to specific stimuli, without a modification in the cell surface expression of integrins [15]. Although a structural change in integrin receptors is possible, the abnormal adhesion may result from an intracellular defect induced by uraemic milieu. In the present study we found a reduced capacity of uraemic CD4+ T lymphocytes to adhere to intact ECM and its immobilized components, FN and LN. Depressed adhesion was not corrected by a non-uraemic environment. These data suggest the possibility of a sustained, acquired intrinsic defect of T cell function in uraemic patients. CAPD patients demonstrated a more pronounced adhesion defect than HD patients. No differences were found between the two groups of patients with regard to several parameters known to affect the immune response in uraemia, as outlined in results. A significant positive correlation was found between serum albumin level and the adherence capacity to ECM and its components. This finding was mainly dependent on the CAPD cohort whose serum albumin was significantly lower than that of HD patients, albeit still in the lower normal range, as reported for most CAPD patients (average albumin levels 3.5 g/dl [19]). A similar observation has recently been made in hyperaluminnemic geriatric patients with normal renal function and a mean serum albumin of 2.7 g/dl (Hershkoviz R. et al, personal communication). The fact that decreased serum albumin adversely affects CD4+ T cell adhesion is in keeping with the well known depressive effect of malnutrition on T cell function [20]. However, our CAPD patients were considered well dialysed with no clinical and laboratory evidence of malnutrition. In support of our in vitro findings is the recent report by Fernandez et al. which documented noticeable differences in the immune response to hepatitis B vaccination in haemodialyzed patients as a result of even minimal changes within normal range of serum albumin [21].

Albumin, per se, may, therefore be an independent variable affecting the immune system. This may explain the reduced adhesive capacity observed in CAPD patients. It may also be the explanation for a diminished adherence of T cells to LN in older CAPD patients (negative correlation), by contrast, an increased adherence was seen in normal subjects and haemodialyzed patients with advancing age. The latter finding is in agreement with the report by Smart et al., in which lymphocytes from aged donors had a higher level of adherence to vascular endothelium [22]. In summary, the results presented herein demonstrate that CD4+ T cells from dialyzed patients exhibit abnormal adhesion properties to ECM, and its major components, FN and LN. The pathogenetic mechanism of this adhesion defect is not mediated via a change in expres-

Fig. 3. The correlation between percentage of adherent cells to fibronectin, laminin and extracellular matrix and serum albumin. ($r = 0.51, 0.51$ and $0.57$, respectively, $*P < 0.01$).
sion of the integrin receptors. It could be due to some conformational change of T cell surface receptors and/or an acquired irreversible cellular defect, both possibly induced by uraemic environment. In support of this theory is our previous finding that a 48 h incubation of normal CD4+ T cells with uraemic sera induced a persistent inhibitory effect on cell adhesion which was not abolished by the removal of the uraemic sera [15].

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References


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