Captopril and losartan modify mitogen-induced proliferative response and expression of some differentiation antigents on peripheral blood mononuclear cells in chronic uraemic patients

Piotr Bartnicki[1], Ewa Majewska[2], Radosław Wilk[1], Zbigniew Baj[2], Jacek Rysz[1]

[1]Department of Nephrology, Hypertension and Family Medicine, Lodz, Poland
[2]Department of Pathophysiology and Immunology, Medical University, Lodz, Poland

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Corresponding author:
Piotr Bartnicki, MD, PhD
Department of Nephrology
Hypertension and Family Medicine
Żeromskiego 113,
90-549 Łódź, Poland
Phone/fax:
+48 42 639 34 65
E-mail:
piotr.bartnicki@neostrada.pl

Abstract

Introduction: The renin-angiotensin system plays an important role in immune mechanisms. It is still insufficiently known whether angiotensin converting enzyme inhibitors and selective angiotensin type 1-receptor antagonists interfere with immune cells. That led us to investigate whether angiotensin converting enzyme inhibitor – captopril and selective angiotensin type 1-receptor antagonist – losartan modify some functions of immune cells in chronic uraemic patients and healthy subjects.
Material and methods: In 15 chronic uraemic patients on regular hemodialysis treatment and 15 control subjects we investigated *in vitro* mitogen induced proliferative response of peripheral blood mononuclear cells and expression of some lymphocyte differentiation antigens in the presence of captopril and losartan, equal to their therapeutic concentrations. As mitogen was used phytohemagglutinin P and phorbol myristate acetate.
Results: In our *in vitro* experiment captopril and losartan, in investigated concentrations, depressed mitogen induced proliferative response of peripheral blood mononuclear cells and lowered expression and numbers of some lymphocyte differentiation antigens in chronic uraemic patients and control subjects.
Conclusions: Our *in vitro* results seem to indicate that captopril and losartan interfere with immune cells and depress some functions of immune cells in chronic uremic patients and healthy subjects. It is very difficult at present to establish significance of these *in vitro* immunosuppresive effects of captopril and losartan reflects their *in vivo* action. Its significance for clinical implications necessitates further extensive studies.

Key words: captopril, losaratan, chronic uraemia, blood mononuclear cells, proliferative response, differentiation antigens.

Introduction

The renin-angiotensin system plays an important role in immune mechanisms [1]. Immune cells were shown to synthesise angiotensinogen [2], angiotensin I (AT I) [3], angiotensin II (AT II) [3, 4] and to express angiotensin type 1 receptors (AT1-R) [5, 6]. The most biologically active AT II is well known as a growth factor [7, 8], it induces production of different cytokines [9, 10] and exerts immunomodulatory effects [11, 12]. Therefore, pharmacological intervention in rennin – angiotensin system should be

expected to affect immune functions. Indeed, mitogen-induced T cell proliferation and the primary antibody response of B cells was suppressed by angiotensin converting enzyme inhibitor (ACEI) – captopril (CAP) *in vitro* in healthy subjects [13, 14]. CAP prevented activation – induced apoptosis in T cell hybridomas by interfering with cell activation signals [15]. Moreover, ACEIs including CAP were found to exert beneficial effects in patients with rheumatoid arthritis [16] and lupus nephritis [17].

It is still insufficiently known whether losartan (LOS) – a selective AT-1 receptor antagonist (AT1-RA) – interferes with immune cells. It was shown only that LOS inhibits formyl-met-leu-phe (fMLP) – stimulated neutrophil shape change, adherence and chemiluminescence response *in vitro* [18, 19] and decreases T cell activities hos patients with primary hypertension [20]. We have found no investigations concerning the influence of ACEI and AT1-RA on the expression of lymphocyte differentiation antigens in chronic uraemia and in healthy subjects. At present it is difficult to establish to what extent chronic uraemia affects renin-angiotensin system. Patients with chronic renal failure have plasma AT II levels similar to those found in control subjects [21].

In this study we investigated whether CAP and LOS, equal to their therapeutic concentrations, modify mitogen-induced proliferative response of peripheral blood mononuclear cells (PBMNC) and expression of some lymphocyte differentiation antigens in chronic uraemic patients and healthy subjects. Our investigated model (uraemic and healthy subjects) could show differences in investigated groups, what could have different clinical implications.

Material and methods

The studies were performed on 15 chronic uraemic patients (7 women, 8 men), aged 37.2 ±2.9 years, on regular hemodialysis treatment for 5.5 ±1.1 years. Four-h hemodialysis sessions were performed three times a week by means of AK-200 machines (Gambro; Lund, Sweden) and polysulfone dialyzers (Diacap LO PS 15; B. Braun Melsungen AG, Germany). Mean predialysis blood serum creatinine concentration was 754.2 ±61.1 μmol/l, urea 24.4 ±2.3 mmol/l and hydrocarbonate 17.1 ±0.8 mmol/l. None of the patients had clinical symptoms of infection, they were HBs-, HCV- and HIV- negative and were not receiving any drugs affecting the immune system and blood transfusion in the last 3 months. In particular, they were not receiving any drugs known to affect renin-angiotensin system. The causes of end-stage renal failure in the uraemic patients were glomerulonephritis (7 patients), interstitial nephritis (6 patients), and polycystic kidney disease (2 patients).

The control group consisted of 15 healthy persons (7 women, 8 men), aged 36.2 ±3.2 years. The study was approved by the Internal Ethics Committee of the Medical University of Łódź and each subject (uraemic patients and healthy) gave informed consent.

Venous blood samples were drawn on a fasting basis at 8.00 a.m., in chronic uraemic patients immediately before hemodialysis. PBMNC were obtained under sterile conditions by centrifugation of blood on a density gradient using Gradisol G (Polfa; Kutno, Poland) [22, 23].

The cells were suspended in RPMI 1640 supplemented with 10% heat inactivated foetal calf serum, penicillin 100 lU/ml and streptomycin 50 μg/ml as a culture medium. The final PBMNC suspension used for further experiments consisted of lymphocytes 88.1 ±2.0%, monocytes 11.0 ±0.9% and neutrophils 0.9 ±0.1%. In all experiments PBMNC were at least 98 ±1% viable as checked by trypan blue exclusion.

Triplicate sterile cultures contained 2×10^5 PBMNC in culture medium with respective mitogen and respective ACEI or AT1-RA. Phytohemagglutinin P (PHA) (Difco; Detroit, USA) 5 μg/ml and phorbol myristate acetate (PMA) (Sigma Chemical Co., St. Louis, USA) 10 μg/ml were used as mitogens. CAP was used as ACEI (Sigma Chemical Co.; St. Louis, USA) in three concentrations: 1 μg/ml (CAP-1), 2.5 μg/ml (CAP-2), 5 μg/ml (CAP-3), i.e. those within blood serum therapeutic ranges [14]. LOS was used as AT1-RA in three concentrations: 0.25 μg/ml (LOS-1), 0.5 μg/ml (LOS-2), 1 μg/ml (LOS-3). These concentrations of LOS are within their blood serum therapeutic ranges in normal and chronic uremic patients [24]. Chiu *et al.* has showed that LOS (DuP 753) is AT1-RA as well *in vivo* as *in vitro* experiments [25].

The triplicate cultures were incubated for 72 h at 37°C in 5% CO_2 and air in a humidified incubator. Eighteen hours before the termination of the cultures 1 μCi of ^{3}H-thymidine ([methyl-^{3}H]-thymidine; UVWR; Prague, Czech Republic, spec. act. 10.2 Ci/mmol) was added. Incorporation of ^{3}H-thymidine into the cells was determined in counts per minute (c.p.m.) using LKB Wallac 1219 scintillation counter (Rack Beta; Turku, Finland). The proliferative responses of the cells were expressed as proliferative indices reflecting ratios of c.p.m. from the cultures containing the cells and mitogen with or without ACEI or AT1-RA to c.p.m. from the respective cultures containing the cells alone.

Sterile PBMNC suspensions (10^6 cells/ml) containing PHA (5 μg/ml) or PMA (10 μg/ml) were incubated with or without CAP in the concentration of 5 μg/ml or LOS in the concentration of 1 μg/ml for 72 h at 37°C in 5% CO_2 and air in a humidified

incubator. After threefold washing with phosphate-buffered saline (PBS) (pH = 7.4), cell pellets were incubated with 10 µl monoclonal antibodies: CD3/RPE + HLA – DR/FITC or CD4/FITC + CD8/RPE DAKO; Glostrup, Denmark) for 25 min at 4°C. After washing with PBS, 400 µl of 1% paraformaldehyde was added to the cell pellets as recommended by Lal *et al*. [26]. Numbers of CD3+, CD4+, CD8+ and HLA-DR+ cells as well as expression of CD3, CD4, CD8 and HLA-DR antigens were determined from the analysis of 5000 cells using FACScan flow cytometer (Becton-Dickinson; Heidelberg, Germany).

Following calculations of arithmetic means (X) and standard errors of the means (SEM), pared and unpaired Student's *t*-tests were used to evaluate the significance of differences. The differences were recognised as significant when $p < 0.05$.

Results

In chronic uraemic patients the low CAP concentration (CAP-1) significantly depressed, while the high LOS concentration (LOS-3) significantly increased proliferative responses of unstimulated PBMNC. In healthy subjects only the high CAP concentration (CAP-3) significantly affected proliferative responses of unstimulated PBMNC (Table I). In both investigated groups CAP and LOS significantly depressed PHA- and PMA-induced proliferative responses of PBMNC (Table I). In chronic uraemic patients expressions of CD3, CD4, CD8 and HLA-DR antigens on PHA- or PMA-stimulated PBMNC were significantly lower as compared with those observed in healthy subjects (Table II). In both observed groups CAP and LOS significantly increased expressions of CD3 antigen on unstimulated PBMNC, but they did not change CD4, CD8 and HLA-DR antigen expression on these cells (Table II). In chronic uraemic patients and in healthy subjects LOS and CAP significantly lowered expressions of CD4 and HLA-DR antigen on the PHA-stimulated PBMNCs, while CD8 antigen expressions remained unaffected (Table II). LOS significantly depressed expression of CD3 and CD4 antigen only on PMA-stimulated PBMNCs in chronic uraemic patients, while in healthy subjects CAP and LOS significantly lowered expression of CD8 antigen on the PMA-stimulated PBMNCs only (Table II).

In chronic uraemic patients numbers of CD3+, CD4+, CD8+ and HLA-DR+ cells following PHA- or PMA- stimulation were lowered as compared with those observed in healthy subjects (Table III). CAP and LOS significantly increased numbers of CD3+ cells of unstimulated PBMNC in both observed groups (Table III). In both investigated groups CAP and LOS significantly lowered numbers of CD3+ and CD4+ cells after PHA stimulation, but they did not significantly change numbers of CD8+ and HLA-DR+ cells. CAP and LOS did not significantly affect numbers of CD3+, CD4+, CD8+ and HLA-DR+ cells after PMA-stimulation in both investigated groups (Table III).

In chronic uraemic patients CD4+/CD8+ ratio for unstimulated and PHA-stimulated PBMNC was significantly lower than those found in healthy subjects, while after PMA-stimulation was similar to those found in healthy subjects (Table IV). LOS significantly lowered CD4+/CD8+ ratio for PBMNC following PHA-stimulation in chronic uraemic patients only (Table IV).

Table I. *In vitro* PHA- and PMA-induced proliferate responses of PBMNC in the presence of various concentrations of CAP or LOS in chronic uraemic patients and healthy subjects. Results are expressed as proliferative indices (X ± SEM) in relation to PBMNC alone

	Chronic uraemics (n = 15)	Healthy subjects (n = 15)
PBMNC + CAP-1	0.6 ±0.1*	1.9 ±0.2
PBMNC + CAP-2	0.8 ±0.1	1.8 ±0.1
PBMNC + CAP-3	1.0 ±0.1	1.7 ±0.1[a]
PBMNC + LOS-1	1.1 ±0.2	1.8 ±0.1
PBMNC + LOS-2	1.2 ±0.3*	1.8 ±0.1
PBMNC + LOS-3	1.8 ±0.5*[a]	1.9 ±0.1
PBMNC + PHA	28.0 ±1.9[a]	81.0 ±4.7[a]
PBMNC + PHA + CAP-1	8.1 ±0.8[b]	30.1 ±2.2[b]
PBMNC + PHA + CAP-2	11.0 ±1.2[b]	41.2 ±31[b]
PBMNC + PHA + CAP-3	29.1 ±2.9*	54.0 ±5.3
PBMNC + PHA + LOS-1	19.1 ±1.6[b]	51.1 ±4.5[b]
PBMNC + PHA + LOS-2	23.5 ±2.1*	72.4 ±6.7
PBMNC + PHA + LOS-3	26.2 ±2.8	87.0 ±7.0
PBMNC + PMA	29.2 ±1.9*[a]	42.1 ±3.1[a]
PBMNC + PMA + CAP-1	12.3 ±1.7*[b]	24.6 ±2.1[b]
PBMNC + PMA + CAP-2	8.1 ±0.8[b]	21.2 ±2.1[b]
PBMNC + PMA + CAP-3	6.3 ±0.7[b]	17.4 ±2.3[b]
PBMNC + PMA + LOS-1	12.4 ±1.4*[b]	17.9 ±1.2[b]
PBMNC + PMA + LOS-2	4.1 ±0.5[b]	18.2 ±1.1[b]
PBMNC + PMA + LOS-3	4.0 ±0.4[b]	14.8 ±0.9[b]

**p < 0.05 in relation to healthy subjects, [a]p < 0.05 in relation to PBMNC, [b]p < 0.05 in relation to PBMNC + PHA/PMA*

Discussion

Our *in vitro* investigations revealed that captopril and losartan in examined concentrations significantly depressed PHA-induced proliferative responses of PBMNC from chronic uraemic patients and control subjects. Inhibitory actions of CAP and LOS seemed to be inversely proportional to their

Table II. Effects of CAP (5 μg/ml) and LOS (1 μg/ml) on in vitro PBMNC differentiation antigen expression measured as fluorescence intensity (X ± SEM) in chronic uraemic patients (n = 15) and healthy subjects (n = 15)

	CD3	CD4	CD8	HLA-DR
Chronic uraemics				
PBMNC	127.1 ±27.9*	48.1 ±7.3*	74.7 ±13.5*	50.4 ±6.9*
PBMNC + CAP	183.9 ±54.4*	59.1 ±10.4*[a]	73.1 ±8.6*	55.3 ±6.1
PBMNC + LOS	157.5 ±40.9*[a]	51.1 ±7.6*	80.8 ±18.6*	51.5 ±4.9*
PBMNC + PHA	157.6 ±39.5*	94.1 ±29.8*	77.6 ±11.7*	61.0 ±10.9*
PBMNC + PHA + CAP	148.6 ±34.5*	51.6 ±8.1*[b]	69.4 ±9.6*	46.5 ±6.3*[b]
PBMNC + PHA + LOS	131.6 ±29.6*[b]	52.1 ±6.9*[b]	64.1 ±8.4*	40.3 ±2.6*[b]
PBMNC + PMA	165.1 ±40.1	50.8 ±8.1*	56.5 ±8.1*	43.2 ±4.1*
PBMNC + PMA + CAP	167.8 ±44.0	60.7 ±12.9*	55.6 ±9.9	41.4 ±4.9*
PBMNC + PMA + LOS	123.0 ±33.4*[b]	36.5 ±6.9*[b]	52.1 ±9.5	63.8 ±2.4
Healthy subjects				
PBMNC	181.0 ±27.7	75.4 ±14.8	138.4 ±49.6	83.7 ±22.1
PBMNC + CAP	263.0 ±63.3[a]	88.1 ±17.4	110.8 ±35.1	69.6 ±16.3
PBMNC + LOS	282.2 ±80.1[a]	99.3 ±17.7	124.0 ±38.4	76.1 ±17.1
PBMNC + PHA	220.6 ±40.1	270.8 ±27.6	111.3 ±25.1	104.6 ±42.4
PBMNC + PHA + CAP	192.9 ±31.8	86.9 ±16.6[b]	116.3 ±27.2	82.7 ±27.3[b]
PBMNC + PHA + LOS	177.3 ±37.7[b]	91.6 ±18.9[b]	115.4 ±25.1	77.4 ±20.1[b]
PBMNC + PMA	219.8 ±41.6	89.5 ±21.8	92.4 ±19.6	72.9 ±19.9
PBMNC + PMA + CAP	211.8 ±43.9	85.4 ±13.2	77.3 ±17.9[b]	70.4 ±18.8
PBMNC + PMA + LOS	222.7 ±41.3	98.9 ±18.6	78.1 ±19.3[b]	69.4 ±18.1

$p < 0.05$ in relation to healthy subjects, [a]$p < 0.05$ in relation to PBMNC, [b]$p < 0.05$ in relation to PBMNC + PHA/PMA

concentrations. When uraemic and normal PBMNC were stimulated with PMA, a protein kinase C activator [27], their proliferative responses were also significantly depressed in the presence of CAP and LOS, but this inhibitory action proved to be directly proportional to their concentrations. This finding seems to indicate that inhibitory effects of CAP and LOS on PBMNC proliferative responses are probably cell membrane-unrelated. The mechanism of CAP and LOS inhibitory actions on PHA- and PMA-induced proliferative responses of uraemic and normal PBMNC remains unclear. A possible mechanism of CAP inhibitory actions is the elevation of prostaglandin E_2 in supernatants in stimulated PBMNC cultures [28] and increase of intracellular cyclic adenosine monophophate (cAMP) content, which inhibits cell proliferation [29]. This prostaglandin mechanism seems to be important, because indomethacin can reverse inhibitory action of CAP on lymphocyte proliferation [28]. The other inhibitory mechanism of CAP is its direct influence on monocytes and macrophages, which play an important role in proliferative responses of lymphocytes [14]. CAP can suppress production of interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) by human PBMNC [9, 10]. As immune cells, especially mononuclear leukocytes, can synthesis angiotensinogen, AT I, AT II and can express AT1-R, one cannot exclude that inhibitory actions of CAP on proliferative response of PBMNC is related to effects of local renin-angiotensin system on monocytes and lymphocytes [30].

At present it is very few investigations about LOS influence on immune cells. Raiden *et al*. observed that LOS significantly inhibited chemiluminescence emission by fMLP-stimulated monocytes [18]. Therefore, it is possible that inhibitory action of LOS on PBMNC proliferate response is due to its influence on monocytes. One cannot exclude that direct binding of LOS to ATl-R on mononuclear leukocytes could affect their immune functions. It was reported that ATl-R are involved in transmembrane signal transduction [31] and that stimulating action of AT II on anti-CD8-antibody induced proliferate response of murine T cells was transduced through ATl-R [32].

In our *in vitro* investigations we have found that CAP and LOS significantly lowered some lymphocyte antigen expressions, especially CD3, CD4 and HLA-DR antigens on PHA-stimulated PBMNCs, and they significantly reduced percentages of CD3+ and CD4+ cells. After PBMNC stimulation with PMA CAP

Table III. Percentages (X ± SEM) of PBMNC expressing differentiation antigens following *in vitro* PHA or PMA stimulation with or without CAP (5 μg/ml) or LOS (1 μg/ml) in chronic uraemic patients (n = 15) and healthy subjects (n = 15)

	CD3+	CD4+	CD8+	HLA-DR+
Chronic uraemics				
PBMNC	23.9 ±2.6*	2.9 ±0.9	10.8 ±1.3*	8.2 ±0.8
PBMNC + CAP	39.8 ±4.9*[a]	2.6 ±0.8*	10.2 ±1.3*	8.6 ±0.9*
PBMNC + LOS	39.7 ±3.5*[a]	3.3 ±1.2	10.5 ±1.5	7.7 ±0.8
PBMNC + PHA	47.0 ±1.8*	15.4 ±3.1*[a]	13.7 ±2.5*	18.8 ±1.7
PBMNC + PHA + CAP	24.6 ±2.6*[b]	12.3 ±3.6	12.9 ±2.0	18.4 ±2.5
PBMNC + PHA + LOS	25.4 ±3.2*[b]	10.6 ±2.3[b]	14.2 ±2.4*	19.0 ±3.0
PBMNC + PMA	34.8 ±4.3*	2.9 ±0.7	7.9 ±1.5	7.1 ±1.5*
PBMNC + PMA + CAP	26.2 ±3.9	4.0 ±0.1	7.8 ±1.5	7.9 ±1.4*
PBMNC + PMA + LOS	24.7 ±3.8	3.1 ±0.8	7.2 ±1.6	7.6 ±1.5*
Healthy subjects				
PBMNC	32.1 ±2.0	6.6 ±2.5	5.9 ±1.4	10.7 ±1.6
PBMNC + CAP	41.5 ±3.3[a]	5.2 ±2.1	7.1 ±1.4	10.8 ±1.7
PBMNC + LOS	46.8 ±2.2[a]	5.4 ±2.3	9.6 ±1.6[a]	9.5 ±2.0
PBMNC + PHA	89.9 ±5.9	29.5 ±2.2	19.8 ±1.4	29.7 ±1.7
PBMNC + PHA + CAP	62.7 ±4.5[b]	17.8 ±2.5	17.9 ±1.6	18.4 ±1.0
PBMNC + PHA + LOS	63.5 ±5.4[b]	15.7 ±2.2[b]	16.5 ±1.8	21.5 ±2.7
PBMNC + PMA	53.2 ±3.1	6.8 ±1.3	18.2 ±1.8[b]	23.1 ±2.5
PBMNC + PMA + CAP	42.2 ±3.7	5.3 ±1.0	17.8 ±1.7	21.6 ±2.0
PBMNC + PMA + LOS	41.8 ±4.3	5.8 ±2.8	18.4 ±2.1	20.2 ±2.1

**p < 0.05 in relation to healthy subjects, [a]p < 0.05 in relation to PBMNC, [b]p < 0.05 in relation to PBMNC + PHA/PMA*

and LOS significantly lowered expression of CD8 antigen in healthy subjects and LOS significantly depressed expression of CD3 and CD4 antigen in chronic uraemic patients. We have, as yet, found no literature data concerning CAP and LOS effects on the PBMNC differentiation antigen expression.

The mechanism of influence of ACEI and selective AT1-RA on transmembrane signal transduction and surface antigen expression during cell activation remains unclear, because molecular events leading to lymphocyte differentiation antigen expression after mitogen-induction have not been elucidated. Edwards *et al*. have reported that ATl-R is involved in transmembrane signal transduction through G-protein, which causes phospholipase C activation, rise of intracellular Ca^{+2} concentration and decrease in intracellular cAMP levels [31]. Among speculative explanations one can take into consideration that direct blockade of ATl-R on immune cells could induce of conformational alterations of membrane receptors on these cells, analogous to those observed by Janeway *et al*. with altered peptide ligands [33], which can modify the cell responses. However, it necessitates further extensive studies.

Table IV. CD4+/CD8+ cell ratios (X ± SEM) following *in vitro* PHA- or PMA-stimulation of PBMNC with or without CAP (5 μg/ml) or LOS (1 μg/ml) in chronic uraemic patients and healthy subjects

	Chronic uraemics (n = 15)	Healthy subjects (n = 15)
PBMNC	0.3 ±0.1*	2.6 ±1.4
PBMNC + CAP	0.3 ±0.1*	1.0 ±0.5
PBMNC + LOS	0.4 ±0.1*	0.8 ±0.4
PBMNC + PHA	2.0 ±0.4	2.0 ±0.4
PBMNC + PHA + CAP	1.5 ±0.6*	2.0 ±0.3
PBMNC + PHA + LOS	1.2 ±0.4*[b]	1.9 ±0.3
PBMNC + PMA	0.7 ±0.3	0.7 ±0.2
PBMNC + PMA + CAP	0.8 ±0.3	0.7 ±0.2
PBMNC + PMA + LOS	0.9 ±0.5	1.0 ±0.4

**p < 0.05 in relation to healthy subjects, [b]p < 0.05 in relation to PBMNC + PHA/PMA*

We have found that chronic uraemic patients' expressions of CD3, CD4, CD8 and HLA-DR antigens on the PHA- and PMA-stimulated PBMNC were significantly lowered in comparison with healthy

subjects. We are not able to exclude that it is due to the existence of some persisting intracellular derangements of the uraemic cells previously exposed to uraemic toxins and metabolic acidosis *in vivo*. Delfraissy *et al*. has reported that CAP induces lymphocyte T suppressor activity [14]. We have found that CAP did not affect CD4+/CD8+ ratios in both groups, but LOS significantly lowered this ratio in PHA-stimulated PBMNC in uraemic patients only.

In conclusion, our *in vitro* investigations, even so the number of examined cases was limited, indicate that ACEI – CAP and selective AT1-RA – LOS could depress mitogen-induced proliferative responses of PBMNC and could lower expression of some lymphocyte differentiation antigens in chronic uraemic patients and healthy subjects.

At present it is very difficult to establish significance of these *in vitro* immunosuppresive effects of CAP and LOS reflects their *in vivo* actions in chronic uremic patients and healthy subjects. However, complete elucidation of mechanisms of these possible immunosuppresive actions of CAP and LOS and its significance for chronic uremic patients necessitates further extensive studies.

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