

# Effects of parathyroid hormone and serum calcium on the phenotype and function of mononuclear cells in patients with primary hyperparathyroidism

H. Kotzmann, M. Köller, C. Abela, M. Clodi, M. Riedl, W. Graninger, B. Niederle and A. Luger

University of Vienna, Vienna, Austria

## Abstract

**Background** Several studies have demonstrated specific influence of parathyroid hormone (PTH) on immune parameters, especially on T- and B-cell function, migration of polymorphonuclear leucocytes (PMNLs) and antibody synthesis, in patients with secondary hyperparathyroidism and chronic renal failure and recently also in patients with primary hyperparathyroidism (pHPT).

**Methods** We therefore examined 12 patients with pHPT before and 6 months after parathyroidectomy (PTX) and nine sex- and age-matched control subjects to determine the impact of PTH and serum calcium concentrations on several immune parameters, including (a) serum concentrations of immunoglobulins, (b) immunophenotype of peripheral blood lymphocytes, (c) phytohaemagglutinin (PHA)-induced lymphocyte proliferation and (d) monocytic surface marker expression.

**Results** Serum concentrations of immunoglobulins (IgG, IgA, IgM) were unaffected by elevated serum PTH and calcium levels. T lymphocytes (CD3), B lymphocytes (CD19), NK cells (CD16/56) and monocytes (CD16) revealed a normal distribution and were not different before and after PTX in patients with pHPT when compared with the control group. CD4+ T-helper lymphocytes were significantly elevated pre- and post-operatively in patients with pHPT. The lymphocyte proliferation response to PHA in the highest concentration ( $12.5 \mu\text{g L}^{-1}$ ) tested was significantly suppressed in patients with pHPT preoperatively when compared with the patients post-operatively and the control group. In addition, both CD4+ and CD8+ lymphocytes showed a lower expression of activation markers, interleukin 2 (IL-2) receptor (CD25) and transferrin receptor (CD71), which could be partially restored 6 months after PTX, but did not reach normal values.

**Conclusion** In summary, in contrast to the findings in patients with secondary HPT, pHPT appears to be associated with less alterations of immune functions. Chronically elevated serum PTH and calcium concentrations in patients with pHPT induce a higher percentage of CD4+ helper T lymphocytes and a suppressed lymphocyte response to PHA as well as a reduced expression of activation markers on peripheral blood lymphocytes.

**Keywords** Hypercalcaemia, immune function, primary hyperparathyroidism

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Division of Endocrinology and Metabolism, Department of Medicine III, Ludwig Boltzmann Institute of Clinical Endocrinology (H. Kotzmann, M. Clodi, M. Riedl, A. Luger); Division of Rheumatology, Department of Medicine III, Ludwig Boltzmann Institute of Clinical Endocrinology (M. Köller, W. Graninger); Department of Surgery, University of Vienna, Austria (C. Abela, B. Niederle).

Correspondence to: H. Kotzmann, Department of Medicine III, University of Vienna, Währinger Gürtel 18–20, 1090 Vienna, Austria.

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## Introduction

After the detection of parathyroid hormone (PTH) receptors on human lymphocytes, recent studies have demonstrated that T and B lymphocytes are target cells for the action of PTH [1]. Most studies have been performed *in vitro* with lymphocytes from patients with chronic renal failure (CRF) suffering from secondary hyperparathyroidism. Elevated PTH levels are thought to cause several immunological defects, including diminished antibody response to several antigens, diminished immunoglobulin production and altered polymorphonuclear leucocyte

**Table 1** Patients' characteristics.

Number	Age	Sex	iPTH (preoperative) (pg mL <sup>-1</sup> )	iPTH (post-operative) (pg mL <sup>-1</sup> )	Ca (preoperative) (mmol L <sup>-1</sup> )	Ca (post-operative) (mmol L <sup>-1</sup> )	Histo (CCA, OCA, CCH*)
1	71	F	86.9	2	2.9	2.30	CCA
2	52	F	357	20.1	3.85	2.21	CCA
3	59	M	428	23.7	2.89	2.15	CCA
4	77	F	76.6	16.3	3.12	2.26	OCC
5	71	F	182	26.4	2.63	2.24	CCA
6	56	M	192	25.7	2.92	2.31	CCA
7	54	M	60.6	5	2.75	2.16	CCH
8	25	F	75.3	15.5	3.22	2.59	CCA
9	35	F	104	30.1	2.73	2.43	CCA
10	65	F	284	45.9	3.6	2.47	CCA
11	49	F	189	34.7	2.61	2.38	CCA
12	57	F	140	36.5	2.85	2.30	CCA

CCA, chief cell adenoma, OCC, oxyphil cell adenoma, CCH, chief cell hyperplasia.

(PMNL) migration [2–5]. In addition to the effects on B-cell function, there is strong evidence that long-term elevation of PTH also causes impaired T-cell function [6]. Besides other uraemic toxins that also might affect immune function in patients with CRF, elevated PTH levels appear to reduce the number of T lymphocytes, decrease the CD4/CD8 ratio and inhibit lectin-induced DNA synthesis by peripheral lymphocytes [6]. The only study dealing with patients with primary hyperparathyroidism (pHPT) also found a decrease in the CD4/CD8 ratio and a significant inhibition of lectin-stimulated lymphocytic transformation [7]. Long-term elevation of PTH concentrations thus seems to be associated with an impairment of the immune system.

On the other hand, complete removal of normal parathyroid glands causes acute involution of the thymus followed by a diminished production of thymic lymphoblasts, and daily injections of PTH can restore serum antibody titers [8,9]. In addition, elevation of PTH concentrations only during a short period stimulates phytohaemagglutinin (PHA)-induced T-cell proliferation and interleukin (IL) 2 production [10].

The aim of the present study was to determine whether immunological abnormalities in patients with pHPT exist and whether these abnormalities could be influenced by successful parathyroidectomy leading to normal PTH and serum calcium.

### Patients and methods

Twelve patients with primary pHPT with a mean age of 56.6 (range 25–77) years were studied before and 6 months after parathyroidectomy, and compared with nine sex- and age-matched control subjects after informed consent was obtained. Characteristics of the patients and the control group are shown in Tables 1 and 2 respectively.

The investigation was performed at 08.00 h after an overnight fast. In the previous 2 weeks there had been no

history of infections, surgery or drugs influencing calcium metabolism or the immune system. Low post-operative calcium levels were corrected only by oral calcium supplementation.

Serum levels of PTH were measured by a commercially available radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The intra-assay and interassay coefficients of variation (CV) were 7.5 and 6.8%.

Immunoglobulin serum levels were quantified by radioimmunodiffusion.

### Immunofluorescence and flow cytometry

Aliquots (100 µL) of heparinized whole blood were stained with different anti-CD monoclonal antibodies (mAbs) for 20 min at room temperature. Red blood cells were then lysed in 1 mL of FACS-lysing solution (purchased from Becton Dickinson, San Jose, CA, USA, containing <50% diethylene glycol, <15% formaldehyde) for 15 min at room temperature then pelleted at 400 × g for 6 min. After one washing 1.5 × 10<sup>3</sup> cells were analysed on a flow cytometer (FACScan, Becton Dickinson).

**Table 2** Control group.

Number	Age	Sex	iPTH (pg mL <sup>-1</sup> )	Ca (mmol L <sup>-1</sup> )
1	35	F	17	2.17
2	27	F	31.5	2.32
3	48	F	21	2.4
4	71	F	28	2.3
5	76	F	34	2.5
6	50	F	36.7	2.45
7	55	M	29	2.3
8	56	M	43	2.4
9	53	M	17	2.35

**Table 3** Immunoglobulin concentrations (mg dL<sup>-1</sup>).

	<i>n</i>	IgM	IgA	IgG
Patients with pHPT				
Preoperatively	12	228.0 ± 23.0	264.1 ± 22.7	1367.5 ± 71.4
Post-operatively	12	210.0 ± 18.1	241.0 ± 25.6	1318.0 ± 76.2
Control subject	9	198.0 ± 15.0	228.0 ± 22.0	1284.0 ± 81.0

For two-colour analysis, cells were stained with fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-labelled anti-CD45 vs. CD14, anti-CD3 vs. CD19, anti-CD3 vs. CD4, anti-CD3 vs. CD8 and anti-CD3 vs. CD16/56 (SIMULTEST IMK plus reagent kit, Becton Dickinson) respectively. Three-colour staining of CD4 and CD8 subtypes was performed with anti-CD45RA, CD45RO, HLA-DR, CD25 (IL-2 receptor) (all purchased from Becton Dickinson) and CD71 (transferrin receptor) (from Immunotech, Marseille, France). Lymphocytes were identified by light scatter characteristics (cell size and granulation) and CD45 expression. Isotype-matched mouse antibodies (IgG 1 and IgG 2) conjugated with FITC, PE or peridinin chlorophyll (PerCP, Becton Dickinson) were used as negative controls. Monocytes (CD19) were stained with anti-CD11b (complement receptor 3), CD16 (Fc-gamma R), CD44 (hyaluronic acid receptor), CD54 (ICAM-1), HLA-DR mAbs (FITC-labelled). Counterstaining was performed using anti-CD14 (PE) mAbs.

### Mitogen-induced lymphocyte proliferation

Peripheral blood mononuclear cells (PBMCs) from patients after parathyroidectomy (PTX) were isolated by density gradient centrifugation with Ficoll-Paque (Pharmacia, Uppsala, Sweden). After two washings, cells were resuspended in RPMI-1640 medium (Sigma, St Louis, MO, USA) containing penicillin (50 U L<sup>-1</sup>) and streptomycin (50 µg mL<sup>-1</sup>) and 10% fetal calf serum (FCS) both from IRH Biosciences, (Lenexa, KS, USA) and 25% autologous serum obtained from the patients pre- and post-operatively and from the control group respectively. PBMCs were seeded on a 96-well microtiter plate (10<sup>5</sup> cells per well) with different PHA concentrations (12.5, 6.25, 3.12, 1.56, 0.75, 0.37 µg mL<sup>-1</sup>) and incubated for 48 h at 37°C in a fully humidified air atmosphere containing 5% CO<sub>2</sub>.

Then, cells were pulsed with [<sup>3</sup>H]-thymidine (1 µCi per well) for further 18 h. Cells were recovered with a cell harvester (1295-001 Cell harvester LKB Wallac, Turku, Finland).

Incorporated activity was measured with a liquid scintillation counter (1205 Betaplate TM, LKB Wallac).

### Statistical analysis

Data are expressed as means ± SEM. Means were

compared using the Student's *t*-test for unpaired data and the differences were considered significant with *P* < 0.05.

### Results

Mean serum concentration of iPTH in the patients with pHPT before surgery was 165.6 ± 38.9 pg mL<sup>-1</sup>, mean serum calcium concentration 3.0 ± 0.09 mmol L<sup>-1</sup>. After PTX iPTH (mean 23.2 ± 3.9 pg mL<sup>-1</sup>) and serum calcium (mean 2.3 ± 0.04 mmol L<sup>-1</sup>) were in the normal range (Table 1).

### Immunoglobulins

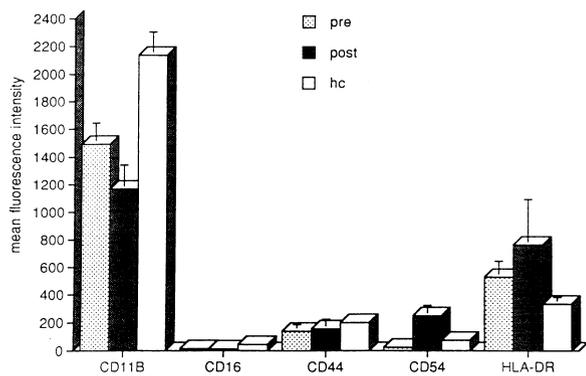
Serum immunoglobulin (IgG, IgM, IgA) levels were found to be within the normal range and did not differ significantly between the patients with pHPT pre- and post-operatively and the control group (Table 3).

### Cell phenotype

The distribution of cell-surface markers for T lymphocytes (CD3+), B lymphocytes (CD19+), NK cells (CD16/56+) and monocytes (CD16+) was evaluated and found to be within the normal range before and after normalization of PTH and serum calcium and did not differ significantly from the control group. However, CD4+ T lymphocytes were significantly elevated and CD8+ T lymphocytes significantly decreased preoperatively in patients with pHPT and were unaffected by normalization of serum PTH and calcium (CD4+: 72.1 ± 2.34% preoperatively and 71.3 ± 2.47% post-operatively vs. 59.17 ± 4.26% in the control group, *P* < 0.007; CD8+: 27.9 ± 1.7% preoperatively and 28.7 ± 1.43% post-operatively vs. 40.83 ± 3.32% in the control group, *P* < 0.006). Thus, the CD4/CD8 ratio was also significantly increased in

**Table 4** Absolute counts of T lymphocytes in patients before and after PTX, and control subjects.

	Pre-PTX	Post-PTX	Control subjects
CD3	1240.9 ± 488.3	1415.9 ± 511.1	1150.3 ± 425.8
CD4	904.6 ± 386.0	1026.6 ± 480.6	680.6 ± 295.7
CD8	336.3 ± 143.7	389.3 ± 117.5	469.7 ± 173.7



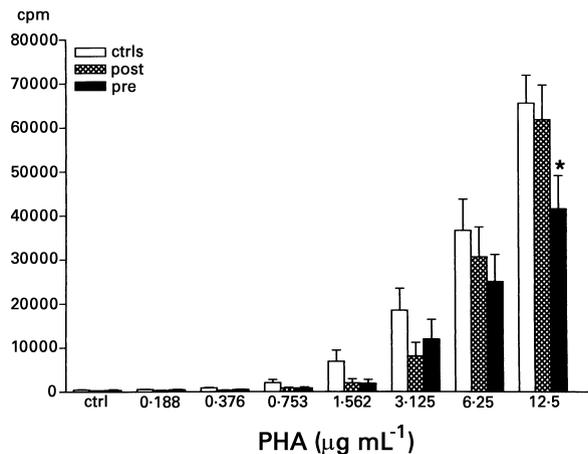
**Figure 1** Relative surface density of different receptors on monocytes. Expression of different activation markers on CD19+ monocytes. [Data from 12 patients with primary hyperparathyroidism (pHPT) pre- and post-operatively and nine healthy control subjects (ctr) are presented as means  $\pm$  SEM.]

pHPT patients. The absolute number of CD3+, CD4+ and CD8+ T lymphocytes is shown in Table 4.

No significant differences in the expression of CD45RA, CD45RO and HLA-DR were found. Expression of CD25 and CD71 in patients with pHPT was decreased pre- and post-operatively in comparison with normal control subjects in CD3+ and CD8+ cells, but the difference was statistically significant only in the CD4+ cells (CD25:  $3.89 \pm 1.17\%$  preoperatively vs.  $12 \pm 3.13\%$  post-operatively,  $P < 0.027$ , and  $26 \pm 2.16\%$  in the normal control subjects,  $P < 0.001$ ; CD71:  $2.22 \pm 0.49\%$  preoperatively vs.  $3.78 \pm 1.08\%$  post-operatively,  $P < 0.008$ , and  $16 \pm 3.77\%$  in the normal control subjects,  $P < 0.003$ ). We also determined receptor density of CD11b (complement receptor 3), CD16 (Fc-gamma Rec), CD44 (hyaluronic acid receptor), CD54 (ICAM-1) and HLA-DR (MHC class II) on monocytes. As all monocytes express these markers, values of mean channel fluorescence are given that reflect receptor density on the cell surface. Relative cell-surface density of CD54 (ICAM-1) and HLA-DR on monocytes (CD16) increased post-operatively with higher values than in the control group, but the difference failed to reach significance (Fig. 1).

### Mitogen-induced cell proliferation

Peripheral blood lymphocytes obtained from pHPT patients post-operatively incubated with autologous serum obtained preoperatively showed a reduced proliferation response to PHA when compared with serum from normal control subjects. In contrast, when the same cells were incubated with serum obtained post-operatively the proliferation response was restored to normal. This phenomenon reached significance only at the highest PHA concentration ( $12.5 \mu\text{g mL}^{-1}$ ) (Fig. 2).



**Figure 2** Mitogen-induced lymphocyte proliferation. Lymphocytes were cultured post-operatively with autologous serum obtained pre- and post-operatively and from healthy control subjects with different concentrations of PHA and proliferation was determined by [ $^3\text{H}$ ]-thymidine incorporation after stimulation by PHA. [Data from 12 patients with primary hyperparathyroidism (pHPT) and nine healthy control subjects (ctr) are presented as means  $\pm$  SEM.]

### Discussion

Many studies in patients with CRF suffering from secondary hyperparathyroidism and one report on patients with primary hyperparathyroidism suggest that PTH plays an important role in the regulation of the lymphatic system [3–8]. Uraemic patients have a higher frequency of infections [11] and neoplasms [12], which is believed to be due to immunological derangements involving both, humoral and cellular immunity. PTH is thought to be one of the principal causes of these alterations. On the other hand, parathyroid hormone is essential for the lymphopoietic activity because parathyroidectomy is followed by thymic atrophy with a decline in thymocyte activity and a decreased proliferation of thymic lymphoblasts [9]. In rats, parathyroidectomy also reduces DNA synthesis and decreases antibody-producing cells [8,9]. In rodents, normal immune response with similar serum antibody titers can be restored after PTX by daily injections of PTH [13].

Studies in CRF patients have shown that elevated serum PTH levels inhibit B-lymphocyte function and proliferation [3,4,14,15]. The addition of PTH to B cells *in vitro* decreases immunoglobulin production [4]. PTH also increases cAMP synthesis, which itself stimulates  $\text{Ca}^{2+}$  entry [16–18], and it is speculated that the inhibitory effect on B-cell proliferation and maturation is due to the enhanced production of cAMP by B cells [14]. In this way, secondary hyperparathyroidism could be responsible for impaired antibody response to various antigens in patients with CRF [2]. In contrast, in our study with patients suffering from pHPT serum immunoglobulin concentrations were within the normal range before and after PTX and similar to an age- and sex-matched control group.

Our results also demonstrate that the lymphocyte transformation response to PHA was suppressed preoperatively. Post-operatively, lymphocyte response was normalized. Klinger *et al.* [10] and Alexiewicz *et al.* [14] have shown in two previous *in vitro* studies that acute exposure to iPTH stimulated PHA-induced proliferation of T cells and IL-2 production in PMNCs of normal subjects in a dose-dependent manner. They speculated that this effect was due to the ability of PTH to enhance the entry of calcium into cells and/or stimulation of protein kinase C. In contrast, the addition of iPTH to PHA did not increase lymphocyte transformation in patients undergoing haemodialysis with secondary HPT [14], and other studies in accordance with our results reported that PTH might inhibit PHA-induced lymphocyte proliferation during a longer incubation period for lymphocytes [6,19]. Calcium overload of the lymphocytes, down-regulation of PTH receptors and the considerably higher PTH levels maintained over a longer period of time in patients with CRF or pHPT might be responsible for the differences of acute exposure to PTH and the results obtained in primary and secondary HPT [20].

In addition to the decreased lymphocyte proliferation, activation markers such as IL-2 receptor (CD25) and transferrin receptor (CD71) were decreased significantly on CD4+ lymphocytes in pHPT patients preoperatively. This decrease could only partially be restored within 6 months.

Two studies [5,7] in patients with primary and secondary HPT demonstrate that the total amount of T lymphocytes and the helper-suppressor ratio was significantly decreased, which contrasts with the stimulatory effect of PTH when PBMLs of normal control subjects were incubated *in vitro* [10]. This decrease in the ratio of helper-suppressor cell was caused mainly by an increase in CD8 suppressor cells [7]. In addition, Angelini *et al.* [5] showed a linear correlation of CD8+ lymphocytes with rising PTH levels and a reverse correlation with total T cells, CD4 and CD4/CD8 ratio. It is of particular interest that all these abnormalities could be restored after PTX [7]. In our patients with pHPT, normal numbers of T (CD3+), B lymphocytes, NK cells and monocytes pre- and post-operatively could be found. However, in contrast to the results of Shasha *et al.* [7], when only three patients were examined in a preliminary study, a significant higher percentage of CD4+ lymphocytes and a reduced number of CD8+ lymphocytes in comparison with an age- and sex-matched control group was detected pre- and post-operatively. We cannot exclude that also in patients with pHPT factors other than elevated PTH and/or calcium concentrations might contribute to these immunological changes as they persist after PTX and normalization of serum PTH and calcium.

Our results of an increase in CD4+ lymphocytes and increased helper-suppressor T-cell ratio in pHPT patients confirm *in vitro* data from normal subjects. These data suggest a stimulatory effect of PTH mainly on T cells. In contrast and in accordance with the observations in patients with secondary HPT, PTH induced a suppression of PHA-induced lymphocyte response and a reduction in

activation markers on CD4+ lymphocytes. Thus, our findings in patients with pHPT are only partially compatible with the ones reported in patients with CRF and secondary HPT. This indicates that factors other than PTH and calcium play a major role in the profound impairment of the immune system in CRF patients.

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