Cancer Immunol Immunother (1997) 45: 109–114

ORIGINAL ARTICLE

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Active and indolent chronic lymphocytic leukaemia – immune and hormonal peculiarities

Received: 19 June 1997 / Accepted: 14 August 1997

Abstract A group of 138 B cell chronic lymphocytic leukaemia (B-CLL) patients, 83 with active disease and 53 having the indolent form of the disease, were evaluated. The aim of the study was to clarify whether indolent and active B-CLL differ in their immune and hormonal characteristics. Peripheral blood lymphocyte proliferation in response to phytohaemagglutinin, concanavalin A, recombinant interleukin-2, dextran sulphate, *Pisum sativatum* agglutinin and wheat germ agglutinin was investigated. Serum immunoglobulin and β_2 microglobulin levels were determined. Adrenocorticotropic hormone (ACTH), cortisol, follicle-stimulating hormone luteinizing hormone, 17β-oestradiol, testosterone, triiodothyronine, thyroxine, thyroglobulin and thyrotropic hormone levels were determined by radioimmunoassay. Active and indolent CLL presented differences in immunological characteristics, as demonstrated by the more severe suppression of T lymphocyte function, reduced IgA level and considerably higher serum β2-microglobulin values in active disease. Immune disturbances were accompanied by hormonal imbalance, depending on disease status: lower ACTH, cortisol and triiodothyronine levels were established to occur in active CLL compared to indolent disease. Male patients demonstrated striking changes in sex hormones, which were more evident in active disease. The findings point to the complexity of immuno-hormonal disturbances in CLL with differences in the active and indolent state of the disease.

Key words Chronic lymphocytic leukaemia · Active/indolent · Immune status · Endocrine hormones

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Indroduction

B cell chronic lymphocytic leukaemia (B-CLL) is a haematological malignancy characterized by proliferation and accumulation of clonal B lymphocytes [20]. Although generally indolent, the clinical course may be fairly aggressive in a subset of patients.

Beside the well-known clinical staging by the Rai [38] and Binet [6] systems, CLL patients are subdivided into those with the indolent and those with the active form of the disease according to the criteria of the Chronic Leukaemia-Myeloma Task Force [9, 10]. B-CLL is characterized by impaired humoral and cellular immune response, which involves defects in the function of T cells or an imbalance of T cell subpopulations, resulting in severe morbidity and even mortality caused by infectious complications [19, 20].

However, it is not clear to what extent immune dysregulation can be connected with the active or indolent status of the disease or vice versa. It has been suggested by Decker et al. [13] that the biological functions of cells are highly dependent on the composition of the surrounding cells. However, in addition to the cells and mediators belonging to the immune system, endocrine hormones have a complex influence on lymphocyte function [5, 25]. It has been proposed that the immune and endocrine systems represent a totally integrated circuit [7].

This study was undertaken with the aim of clarifying whether the indolent and active forms of CLL differ in their immunological and hormonal characteristics.

Materials and methods

Patients

CLL patients were diagnosed after undergoing a complete physical examination, a peripheral blood count, a bone marrow aspiration and biopsy and an immunophenotyping of the peripheral blood or bone marrow lymphocytes. Criteria for diagnosis of CLL were chosen according to the International Workshop on CLL (IWCLL) [28] and

Table 1 Serum immunoglobulin (*Ig*) concentration and β_2-microglobulin (β_2M) levels in chronic lymphocytic leukaemia (*CLL*) patients and controls

Constituent	Mean serum concentration ± SD		*P*
	CLL $n = 138$	Control $n = 38$	
IgG (g/l)	12.7 ± 5.3	17.7 ± 1.1	0.000
IgA (g/l)	1.3 ± 0.8	2.0 ± 0.3	0.000
IgM (g/l)	1.3 ± 0.5	1.2 ± 0.2	0.483
β_2-M (mg/l)	8.0 ± 5.6	1.5 ± 0.7	0.000

Table 2 Serum immunoglobulin (*Ig*) and β_2-microglobulin (β_2M) values in active and indolent CLL

Constituent	Mean serum concentration ± SD		*P*
	Active $n = 3$	Indolent $n = 55$	
IgG (g/l)	12.3 ± 5.5	13.7 ± 0.9	0.220
IgA (g/l)	1.2 ± 0.7	1.7 ± 0.9	0.001
IgM (g/l)	1.3 ± 0.5	1.4 ± 0.5	0.494
β_2-M (g/l)	9.4 ± 4.2	5.6 ± 3.3	0.000

the National-Cancer-Institute – sponsored Working Group Guidelines [8].

Patients were categorized as having active or indolent disease according to the guidelines of the Chronic Leukaemia Working Group [7]. A total of 83 patients appeared to belong to the group with active disease and 55 had the indolent form of CLL. The ratio of men to women was 53/30 for active disease and 35/20 for the indolent form. The mean age of the patients was 62.9 ± 8.7 years; 38 age- and sex-matched healthy subjects, taking no medicaments, served as controls. Patients and control persons gave their informed consent before the investigations. The study was approved by the Ethics Committee of Tartu University.

Serum immunoglobulin concentrations were measured by radial immunodiffusion [33].

Serum β_2-microglobulin levels were assessed by radioimmunoassay [3, 32].

Lymphocyte proliferation

Lymphocyte transformation and proliferation induced by mitogens are standard tools to assay immunocompetence. Mitogen stimulation of peripheral blood lymphocytes was induced by phytohaemagglutinin (PHA; Difco laboratories, Detroit) and concanavalin A (ConA; Sigma, St. Louis) as T-lymphocyte mitogens, and dextran sulphate (DxS; Pharmacia, Fine Chemicals, Uppsala) was used for the stimulation of malignant B lymphocytes [27]. Recombinant interleukin-2 (IL-2; Immunotech, France), wheat germ agglutinin (WGA; E-Y Lab. Inc. San Mateo, Calif. USA), *Pisum sativatum* agglutinin (PSA; St. Louis) were used to stimulate the lymphocytes.

A 20-ml sample of peripheral blood was obtained and anticoagulated in preservative-free heparin at 9.00 a.m. To obtain a closer approximation to the natural biological milieu, we used an optimized whole-blood method to investigate the mitogenic lymphocyte proliferation [14]. Cells were suspended at concentration of 1.0×10^6 cells/ml in RPMI-1640 medium and supplemented with 2 mM glutamine, 50 µg/ml gentamicin and 10 % fetal calf serum. Cells were cultured in the presence of medium alone (control) or mitogens (separately with PHA, ConA, IL-2 and DxS). All assays were performed in triplicate. Cell cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 84 h and pulsed with 1 Ci [^{3}H] thymidine. Proliferation was measured by [^{3}H]thymidine uptake during the last 16 h of culture. Autoradiographic and morphological assessments of proliferating cells were used. Results were expressed as a stimulation index: SI=[mean percentage of proliferating cells with stimulant] / [mean percentage of proliferating cells without stimulant (control)].

Table 3 Mitogen-stimulated proliferative response of peripheral blood lymphocytes in CLL and control group. *PHA* phytohemagglutinin, *PSA Pisum sativatum* agglutinin, *ConA* concanavalin A, *WGA* wheat germ agglutinin, *DxS* dextran sulphate, *IL-2* interleukin-2

Mitogen	Mean stimulation index ± SD		*P*
	CLL $n = 138$	Control $n = 38$	
PHA	3.2 ± 3.0	8.6 ± 2.9	0.000
ConA	3.75 ± 5.6	7.5 ± 3.9	0.009
DxS	5.2 ± 5.7	1.7 ± 1.4	0.002
IL-2	5.1 ± 1.7	3.7 ± 3.2	0.000
PSA	5.3 ± 4.9	1.9 ± 0.9	0.003
WGA	4.7 ± 3.9	1.5 ± 0.7	0.001

First, dose-titration experiments were carried out in selected cases to determine the optimal concentrations of PHA, ConA, IL-2, DxS, PSA, WGA for the stimulation of lymphocytes. PHA was used in concentrations of 5, 10 and 20 µg/ml. Since 10 µg/ml and 20 µg/ml gave similar results, further experiments were performed with a concentration of 10 µg/ml. ConA was used in doses of 1, 2, 5 and 10 µg/ml; the optimal concentration appeared to be 5 µg/ml. Recombinant IL-2 doses tested were 10, 20, 50, 100 and 1000 U/ml and optimal proliferation of lymphocytes was obtained with 100 U/ml IL-2. The optimal dose of 5 µg/ml was established for DxS from dose titrations of 2, 5, 10 and 100 µg/ml. WGA and PSA were used at an optimal dose of 5 µg/ml for stimulation of peripheral blood lymphocytes.

Cell viability, determined by trypan blue exclusion, was more than 96%.

Hormones

Serum for the detection of hormone concentrations was obtained at the same time as the plasma for immunological investigation.

Serum adrenocorticotropic hormone, cortisol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, 17β-oestradiol, triiodothyronine, thyroxine, thyroglobulin and thyrotropic hormone levels were determined by radioimmunoassay [3]. All experiments were performed in triplicate.

Statistical methods

The data reported in the tables were analysed by paired *t*-test. Significance was considered to be at $P<0.05$, but in many situations lower *P* values were obtained, as noted.

Results

Immunological data

Table 1 reflects the significant decrease of IgG and IgA serum concentrations in CLL patients compared to the control group. However, a strikingly higher β_2 microglobulin level was identified in the CLL group.

When CLL active and indolent groups were compared, a higher IgA level was observed in the indolent disease group, also the β_2-microglobulin concentration was different, being lower in patients with indolent CLL (Table 2).

Table 4 Lymphocyte proliferation in response to stimulation with mitogens. *PHA* phytohemagglutinin, *PSA P. sativatum* agglutinin, *ConA* concanavalin A, *WGA* wheat germ agglutinin, *DxS* dextran sulphate, *IL-2* interleukin-2

Mitogen	Mean stimulation index ± SD		*P*
	Active CLL *n* = 83	Indolent CLL *n* = 55	
PHA	2.2 ± 2.0	4.0 ± 3.5	0.036
ConA	3.3 ± 3.1	4.5 ± 3.2	0.261
DxS	5.6 ± 2.9	5.0 ± 2.4	0.620
IL-2	4.9 ± 2.9	5.5 ± 2.1	0.569
PSA	5.4 ± 2.6	5.1 ± 2.7	0.743
WGA	4.9 ± 2.7	4.6 ± 2.5	0.744

Table 5 The values of serum hormones in the of patients with CLL and control group. *ACTH* adrenocorticotropic hormone

Hormone	Mean serum concentration ± SD		*P*
	CLL *n = 138*	Control *n* = 38	
ACTH (ng/ml)	32.5 ± 23.9	38.1 ± 35.8	0.371
Cortisol (nmol/l)	780.7 ± 472.2	425.6 ± 179.1	0.000
Triiodothyronine (nmol/l)	2.0 ± 0.5	1.7 ± 0.4	0.858
Thyroxine (nmol/l)	127.2 ± 37.1	103.5 ± 27.7	0.001
Thyroglobulin (nmol/l)	35.6 ± 29.7	48.8 ± 39.9	0.315

Table 6 The values of serum hormones (ACTH, cortisol, triiodothyronine, thyroxine and thyroglobulin) in active and indolent CLL

Hormone	Mean serum concentration ± SD		*P*
	Active *n* = 83	Indolent *n* = 55	
ACTH (ng/ml)	29.4 ± 17.0	38.5 ± 16.2	0.034
Cortisol (nmol/l)	604.6 ± 410.7	977.5 ± 470.0	0.004
Triiodothyronine (nmol/l)	1.3 ± 0.5	4.5 ± 1.6	0.001
Thyroxine (nmol/l)	131.0 ± 40.9	120.2 ± 28.9	0.109
Thyroglobulin (nmol/l)	32.0 ± 20.6	42.7 ± 27.9	0.391

In Tables 3 and 4, the peripheral blood lymphocyte proliferative response to different mitogens and stimulants is presented. A considerably decreased stimulation index was found for the two T lymphocyte mitogens (PHA and ConA) in the CLL group, compared to healthy persons. However, when active and indolent groups were compared (Table 4), patients with indolent disease demonstrated a higher lymphocyte proliferative response to PHA than did those with active disease. No differences were seen in the stimulation with the second T lymphocyte mitogen (ConA) between the groups of active and indolent CLL. Dextran sulphate stimulated more lymphocytes in the CLL group compared to the controls (Table 3). A similar lymphocyte stimulation index was identified in active and indolent CLL (Table 4).

Il-2, PSA and WGA produced a striking stimulation of peripheral blood lymphocytes in CLL compared to the control group (Table 3). All these three agents induced comparable lymphocyte stimulation in active and indolent CLL patients (Table 4).

Table 7 Concentrations of sex hormones in the serum of patients with CLL and control group (*M* males, *F* females). *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *E* 17β-oestradiol

Hormone	Mean serum concentration ± SD		*P*
	CLL	Control	
FSH (1 μ/l)			
M	22.4 ± 13.2	2.4 ± 1.1	0.000
F	44.1 ± 26.8	35.8 ± 14.4	0.273
LH (1 μ/l)			
M	17.5 ± 9.9	3.2 ± 1.2	0.00
F	32.1 ± 22.1	35.6 ± 14.5	0.596
T (n mol/l)			
M	14.9 ± 12.6	19.2 ± 9.1	0.216
F	2.5 ± 2.2	1.9 ± 0.7	0.618
E (n mol/l)			
M	185.9 ± 124.0	108.45 ± 39.2	0.020
F	199.0 ± 188.9	227.2 ± 107.1	0.601
E/T			
M	17.6 ± 9.14	8.9 ± 6.8	0.003
F	115.0 ± 121.1	131.3 ± 81.0	0.650

Table 8 Concentration of sex hormones in the serum of male patients with active and indolent CLL. *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *E* 17β-oestradiol

Hormone	Mean serum concentration ± SD		*P*
	Active *n* = 83	Indolent *n* = 55	
FSH (1 μ/l)	25.3 ± 8.1	18.3 ± 17.8	0.035
LH (1 μ/l)	18.6 ± 6.3	15.9 ± 7.3	0.004
T (n mol/l)	14.4 ± 13.2	15.6 ± 7.8	0.689
E (n mol/l)	156.5 ± 93.5	229.0 ± 151.8	0.019
E/T	16.5 ± 8.3	19.6 ± 9.9	0.029

Table 9 Concentration of sex hormones in the serum of female patients with active and indolent CLL. *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *T* testosterone, *E* 17β-oestradiol

Hormone	Mean serum concentration ± SD		*P*
	Active *n* = 83	Indolent *n* = 55	
FSH (1 μ/l)	43.6 ± 29.8	45.4 ± 18.9	0.831
LH (1 μ/l)	32.3 ± 25.8	31.9 ± 13.4	0.957
T (n mol/l)	2.6 ± 4.9	2.1 ± 0.8	0.721
E (n mol/l)	171.2 ± 167.5	269.6 ± 225.5	0.100
E/T	104.3 ± 91.9	144.0 ± 120.7	0.305

Hormones

Table 5 presents the concentrations of hormones in CLL compared to those in healthy persons. Cortisol and thyroxine levels were significantly higher in CLL patients. Interestingly, the ACTH serum concentration was higher in the indolent group as was the cortisol level also (Table 6). While thyroxine and thyroglobulin concentrations were the same for active and indolent CLL, the triiodothyronine concentration was found to be significantly higher in indolent CLL patients (Table 6).

Table 7 reflects the sex hormone concentrations in the serum of CLL patients and healthy persons. Here we have observed the differences in male and female patients. Male patients demonstrated considerably higher FSH, LH and 17β-oestradiol levels and oestrogen-to-androgen ratios. No differences were found in the sex hormonal balance in women. When CLL patients with active disease were compared to those with indolent disease (Table 8), male patients with active disease showed higher FSH, LH and lower 17β-oestradiol and oestrogen-to-androgen ratios. No differences were seen in the group of female patients with active and indolent disease (Table 9).

Discussion

B-CLL shows different clinical behaviour, ranging from a controlled state to a progressively malignant condition. The data concerning the validity of immunological parameters for the prognosis of CLL are contradictory.

Comparing the active and indolent status of CLL we have observed differences in immune characteristics. While there was a low level of immunoglobulins, in indolent CLL there was still a tendency for augmentation of IgA concentration compared to active disease. This agrees with the results of Baldini et al. [1]. However, the meaning of this difference is not known.

Demonstrating higher β_2-microglobulin levels in CLL patients and identifying considerably higher concentration of β_2-microglobulin in active CLL, we can agree with the studies that have shown that β_2-microglobulin, although a non-specific molecule, could behave as a reliable "tumor marker" of lymphoproliferative disorders [41]. We can now add the finding that increase of β_2-microglobulin may indicate the activation of CLL and therefore be of some diagnostic importance in distinguishing active and indolent states of CLL and perhaps indicating the necessity for a change of treatment strategies.

Both PHA and ConA induce a low lymphocyte proliferative response in CLL patients. However, T lymphocyte proliferation in response to PHA was significantly lower in active disease. We were not able to corroborate the data of Fernandez et al. [18], who demonstrated that T lymphocytes from CLL patients with active disease have a significant reaction against autologous B cells whereas, in the indolent state, such a reaction was not shown. The response of peripheral blood lymphocytes to the second T cell mitogen, ConA, was significantly different when the groups with active and indolent disease were compared. As PHA and ConA predominantly stimulate different subpopulations of T lymphocytes, our data show evidence for the imbalance of T lymphocyte subpopulations and the functional defects of the cells, as has also been suggested by others [37]. The results described above suggest that the T-helper cell function is more altered in the active form of CLL. Whether this is the outcome of disease activation, or conversely a disturbance that changes indolent to active disease, is not clear. Interestingly, the DxS lymphocyte stimulation index was significantly increased in CLL. It is known that DxS preferentially stimulates undifferentiated subpopulations of normal B lymphocytes [23, 31] and is mitogenic for leukaemic B lymphocytes [17, 29]. Our results possibly indicate that the DxS-induced lymphocyte proliferation reflects the reaction of a malignant population of B cells. We were expecting to find differences between the active and indolent status in the lymphocyte response to DxS, but were not able to confirm this. IL-2 is mitogenic to a relatively small population of IL-2-receptor-positive blood lymphocytes [42]. This is in accordance with our results, when we compare the PHA- and IL-2-induced mitogenesis of normal lymphocytes [PHA SI=8.6 (2.9); IL-2 SI = 3.7 (3.2)].

Data from the present study demonstrate a considerably augmented IL-2 lymphocyte stimulation index in CLL both in the active and indolent state. For CLL it has been demonstrated that leukaemic cells (B cells) have receptors for IL-2 [30, 31] and they can proliferate in the presence of exogenous IL-2 [31, 36]. As CLL cells spontaneously die when cultured *in vitro* [12] it is likely that the *in vitro* malignant lymphocyte accumulation is due to cytokines and growth factors, recently renamed viability factors by Sachs and Lotem [40]. These viability factors prevent CLL neoplastic cells from being triggered for programmed cell death [34]. Control of cell proliferation and death in CLL is sustained by a complex network of cytokines which include interleukin-2 [22, 27, 39]. Our data showing considerable IL-2-induced lymphocyte proliferation in CLL do not agree with Totero's [43] experience, who did not demonstrate the proliferative response of CLL B cells. It is possible that we were able to observe the above-mentioned response because we used whole blood cells (without separating the different lymphocyte subpopulations) which makes the method more comparable with the *in vivo* status. We have demonstrated the DxS induced stimulation of B lymphocytes involved in the malignancy. This is the same population that responds when stimulated with IL-2.

The two lectins – PSA and WGA – have not been reported to be mitogenic for lymphocytes, but our results demonstrated them to be mitogenic for CLL lymphocytes. Lectins are defined as proteins or glucoproteins of non-immune origin, which bind specifically to carbohydrate residues. Neoplastic cell transformation is associated with an altered composition of the carbohydrate of plasma membranes [35, 44]. The mitogenicity of PSA and WGA that we observed may reflect the proliferation of the malignant CLL population irrespective the active or indolent state of the disease.

The data from our immunological investigations allow us to suggest that the behaviour of T and B lymphocytes differs in active and indolent CLL.

The peculiarity of the two distinct states of CLL is further emphasized by hormonal differences between them. As is generally accepted, the immune and endocrine system are interrelated [5, 7]. It has been postulated that one immunoregulatory circuit that links neuroendocrine structures and the immune system is the hypothalamic-pituitary-adrenocortical axis (HPA) [2]. From previous work we

know that the end-product of the HPA axis, cortisol, has multifaceted effects on the immune system. The fluctuations of the endogenous level of blood cortisol are relevant for the continuous endocrine surveillance of the immune cell network [14]. So overall activity of the immune system, and consequently the degree of autoregulatory interactions of immune cells, can therefore be influenced by endogenous cortisol blood levels. One function of the HPA immune circuit is to control and prevent the excessive expansion of lymphoid cells during the immune response. It is conceivable that this circuit, by impeding a cumulative excessive expansion of lymphoid and accessory cells, plays a role in preventing lymphoproliferative diseases.

There is some evidence that imbalances in the immune-HPA axis may be involved in disease state [4]. Our data support this. Significantly elevated cortisol levels in CLL patients, and differences in the ACTH and cortisol levels of patients with active and indolent CLL, have been established. We can suggest that cortisol, as a terminal product of the HPA axis, could have an impact on determining the functional state of CLL. The role of HPA may be important for two reasons: first dysfunction of HPA makes possible uncontrolled lymphoproliferation. At the same time, excessive secretion of cortisol may also influence the immune status – several immune functions can be affected, which has also been demonstrated by our work. We showed that suppression of T cell function can be, at least partly, caused by HPA axis dysfunction. However, higher cortisol levels can, to some extent, control the lymphoproliferation in CLL, as patients with indolent CLL have demonstrated more elevated cortisol levels and a lower lymphocyte count than those with active disease.

The immediate products of activating the HPA axis, i.e. ACTH, may also be immunomodulators, and we have observed higher ACTH levels in the group of patients with indolent CLL.

The hypothalamic pituitary thyroid (HPT) axis can also play a role in regulating the immune response, and thyroid hormones seem to be involved [26]. We have observed changes of thyroid hormone concentrations in CLL. Higher thyroxine levels were found in CLL patients when no changes in thyroglobulin and TSH values were observed. Interestingly, a lower triiodothyronine level was observed in patients with active CLL compared to those with indolent disease.

It was reported earlier that triiodothyronine production from thyroxine is changed in several acute and chronic illnesses [24]. Such abnormality of serum thyroid hormone levels is called “euthyroid sick syndrome” and is now considered to be mediated, at least in part, by the effects of cytokines on the HPT axis [25]. We can, therefore guess that differences in thyroid hormone levels between active and indolent disease reflect the changed effect of immune function on the HPT axis. On the other hand, thyroid hormones have been shown to be important for the normal functioning of lymphoid cells. However, the relationship between the HPT axis and the regulation of the immune system remains currently unknown.

We are beginning to understand the alterations of immune/hormonal interrelations, in active and indolent CLL.

In addition, the imbalance of sex hormones evident in male CLL patients, mentioned above, was more expressed in active disease.

Differences in certain immune responses between men and women have been known for many years [15]: women demonstrate better immune capabilities than men [11]. Functional studies have established a marked effect of sex steroids on events involved in the immune response [21]; however, the exact mechanisms have not been elucidated.

The central targets for sex hormones actions are T cells. Oestrogens depress most, if not all, the major functions of cell-mediated immunity [24]. They may act directly by affecting the target lymphocytes (T-suppressor cells, which possess oestrogen receptors) and indirectly by releasing or inhibiting the secretion of hormones from the thymic reticuloendothelial cells. Grossman [25] suggests that the ratio of oestrogens to androgens may determine whether the circulating hormones will be immunostimulatory or immunoinhibitory. As to FSH and LH, their considerable augmentation in male CLL patients is an interesting observation but difficult to explain. FSH and LH levels are elevated with age, but CLL patients have demonstrated a striking increase when compared to age-matched controls.

As men and women normally have a dimorphic immune response, it is reasonable to suggest that, in CLL immune regulation is altered in different ways in men and women. Sex hormone imbalance in lymphoproliferative malignancies could account for this. Futhermore, the changes in sex hormone balance, through the effect on immune regulation, may be responsible for the worse prognosis of CLL in male patients compared to female.

Our data, taken together, allow us to conclude that:

1. Active and indolent CLL presents differences in immunological characteristics, as demonstrated by more remarkable suppression of T lymphocyte function, reduced IgA concentration and considerably higher serum β_2 microglobulin value in active disease.
2. Immune disturbances can be accompanied by hormonal imbalance, depending on the disease status: lower ACTH, cortisol and triiodothyronine levels have been established in active CLL compared to indolent disease. Sex hormone changes, found only in male patients, are more pronounced in active disease.

The results we have presented provide evidence for the complexity of immunohormonal changes, and that these differ in active and indolent CLL. Whether these differences between the two disease states are a cause or effect of tumour progression is not unequivocally answered.

Further studies are needed to allow definite conclusions concerning immune and hormonal imbalance and its role in the pathophysiology and progression of CLL.

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