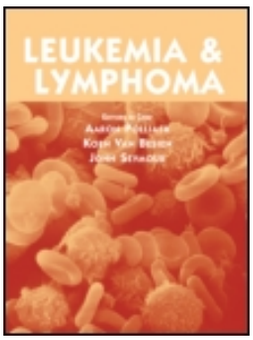

Leukemia & Lymphoma

ISSN: 1042-8194 (Print) 1029-2403 (Online) Journal homepage: http://www.tandfonline.com/loi/ilal20

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To cite this article: Hele Everaus (1992) Hormones and Immune Responsiveness Chronic Lymphocytic ieukemia, Leukemia & Lymphoma, 8:6, 483-489, DOI: 10.3109/10428199209051031

To link to this article: http://dx.doi.org/10.3109/10428199209051031

Published online: 01 Jul 2009.

Leukemia and Lymphoma, Vol. 8, pp. 483–489
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Hormones and Immune Responsiveness in Chronic Lymphocytic Leukemia

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(Received 6 May 1992)

Chronic lymphocytic leukemia (CLL) is a B cell disorder with multiple abnormalities in T-cell function. The status of the immune system will depend to some extent upon the net effect of the changes in the equilibrium of various hormones. In order to investigate the association of the defects in the cellular immunity and hormonal dysregulation in CLL, studies were performed in 130 CLL patients with that disorder. Decreased lymphocyte proliferation in response to mitogen stimulation appears to be an early event in CLL pathogenesis and is not always influenced by the clinical stage of the disease or the specific treatment. The dysfunction of T-lymphocytes was accompanied by increased serum cortisol (C) concentrations. Elevated levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), 17 β-estradiol (E) and testasterone (T) ratio were found in male CLL patients, but not in female patients.

In view of our findings, it is conceivable that there are a number of disturbances in the lymphocyte-microenvironmental regulation, which may be responsible for immuno-hormonal imbalance in some patients with CLL.

KEY WORDS: Cellular immunity hypothalamo-pituitary-adrenal axis sex hormones chronic lymphocytic leukemia

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common lymphoproliferative disease among the elderly. The disease is accompanied by multiple abnormalities of the immune system, including hypogammaglobulinaemia[1], and alterations in T cell phenotype and function[2–6].

As the complexity of immune system has become increasingly clear, it is apparent that it interacts with other systems, particularly the endocrine and nervous systems. A growing body of evidence has accumulated and shown that hormones can modulate immune system functions[7]. One hormonal complex which exerts profound influences on the immune system is that of the hypothalamic-pituitary-adrenal cortical axis (HPA)[8,9], while significant effects on the immune system are also mediated by sex steroids[10].

However, to the best of our knowledge the possibility that the immune defects and hormonal dysregulation seen in CLL could be interrelated has not been previously considered.

This study was undertaken with the aim of clarifying whether the defects in cellular immunity of CLL patients are accompanied by hormonal imbalance. Comparative studies were performed in different clinical stages of CLL defined according to Rai *et al.*[11] and the results obtained in untreated CLL patients were compared with those obtained from treated CLL patients who had received specific therapy.

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MATERIALS AND METHODS

Patients

One hundred and thirty patients with CLL (mean age 62.9 ± 8.7(M ± SD)) were included in this study. The diagnosis and staging of CLL were established by standard clinical criteria[11], according to the clinical staging system of Rai *et al.*[11]. Forty five patients had stage I, 48 stage II and 37 stage III + IV. The male:female was 15:11. Fifty six patients had not received any treatment prior to the study, whereas 74 had been treated with cytotoxic agents. Informed consent was obtained from all patients prior to entry into the study. Twenty nine age and sex matched normal subjects, taking no medications, served as controls.

Lymphocyte proliferation

Heparinized blood (20 ml) was obtained by venipuncture at 9 a.m. Lymphocyte response to mitogen stimulation is a direct measure of in vitro lymphocyte activity and a correlate of cell-mediated immunity. In order to approximate more closely the natural biologic milieu, we used an optimized whole blood method to study the mitogenic lymphocyte proliferation[12]. Cells were suspended in RPMI-1640 and adjusted to a final concentration 1.0×10^6 cells/ml. The cells were cultured in the presence of medium alone (control) or phytohemagglutinin (PHA-Difco, 100 μg/ml) or concanavalin A (Cona-Sigma, St. Louis, 5.0 μg/ml). All cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere for 84 hours, pulsed with ^3H thymidine. Proliferation was measured by ^3H thymidine uptake during last 16 hours of culture. Autoradiographic and morphologic assessment of proliferating cells was used. Results were expressed in mean stimulation index = mean % of proliferating cells with PHA (or Con A)/mean % of proliferating cells without mitogens (control).

Hormones

Plasma adrenocorticotropic hormone and serum cortisol, follicle stimulating hormone, luteinizing hormone, testosterone and 17 β-estradiol concentrations were determined by a radioimmunoassay[13–15]. The following radioimmunoassay kits were used: ACTHK-PR from ORIS Industrie S.A.; ELSA 2-FSA and LH from ORIS Industrie S.A.; STERON-K^{125}-I-M Minsk; STERON-K^{125}-I-M Minsk; STERON-T^{125}-I Minsk.

Statistical Methods

The data reported in the tables were analysed by the paired T test. Significance was considered to be at P 0,05, but in many situations lower P values were obtained as described.

RESULTS

Lymphocyte proliferation in response to PHA and Con A

Tables 1 and 2 reveal PHA and ConA responsiveness of CLL peripheral blood lymphocytes (PBL) compared to healthy persons. The proliferative response of CLL PBL to PHA and Con A was significantly lower than in controls. Depressed PBL proliferation to PHA and Con A have been evaluated already in early stages of CLL (Table 1). Interestingly no statistically significant changes in PBL proliferation to mitogens were found in the different clinical Rai

Table 1 Mitogen stimulated proliferative response of peripheral blood lymphocytes.

Group	*Mean stimulation index ± SD*	
	PHA	*ConA*
Control	8.75 ± 7.63	7.47 ± 7.95
CLL	3.16 ± 3.96	3.75 ± 5.55
	p = 0.000	p = 0.009
Control	8.75 ± 7.63	7.47 ± 7.95
CLL I UTr	3.3 ± 4.03	4.49 ± 6.72
	p = 0.000	p = 0.07
CLL I	3.23 ± 4.32	3.86 ± 6.31
CLL II	3.16 ± 3.43	3.68 ± 5.26
CLL III	3.10 ± 4.28	3.70 ± 5.04
	No Statistically significant differences	

Table 2 Mitogen stimulated proliferative response of peripheral blood lymphocytes in treated (Tr) and untreated (UTr) CLL patients.

Group	*Mean stimulation index ± SD*	
	PHA	*ConA*
CLL I Tr	2.94 ± 2.64	2.78 ± 2.72
CLL I UTr	3.36 ± 4.93	4.34 ± 7.38
	p = 0.768	p = 0.448
CLL II Tr	3.24 ± 3.75	3.23 ± 3.94
CLL II UTr	2.95 ± 2.55	4.77 ± 7.66
	p = 0.797	p = 0.361
CLL III Tr	2.85 ± 4.79	3.38 ± 5.79
CLL III UTr	3.76 ± 2.62	4.54 ± 2.15
	p = 0.572	p = 0.547

stages of CLL. PHA and ConA induced proliferation was similar in both treated (Tr) and untreated (Tr) CLL groups (Table 2).

Hormones

Adrenocorticotropic hormone and cortisol

Mean plasma ACTH levels were within the normal range in CLL patients, whereas cortisol levels were significantly higher in the letter group (Table 3). Higher C values were found in stage I CLL patients (Tables 4, 5) but there were no significant differences in C and ACTH concentrations between the treated and untreated CLL patients (Tables 6, 7, 8).

Follicle stimulating hormone, luteinizing hormone, testosterone and 17 β-estradiol

Values of circulating serum levels of FSH, LH and E in men (M) with CLL were significantly higher than in healthy men (Table 3). However, there were no significant differences in FSH, LH, E and T mean concentrations when women (W) with CLL were compared with the control groups. The E:T ratio was considerably higher in men with CLL, and the highest values were found in the stage I patients (Table 5). Interestingly 17 β-estradiol average concentration in female patients with stage III CLL was significantly lower than in the stage I and II CLL patients, as was the E:T ratio (Table 5). Male CLL patients demonstrated increased FSH, LH and E levels in the early stages of CLL (Table 4).

Data of serum levels of FSH, LH, E and T in treated and untreated CLL patients are given in Tables 6, 7 and 8. The only difference in the stage I was the higher T value of treated male patients compared to untreated male patients. In stage II treated male patients demonstrated higher FSH, LH and lower T values and higher E to T ratio as compared to corresponding untreated group (Table 7). In stage III CLL no differences in mean values of the hormones were found between both treated and untreated groups (Table 8).

Table 3 Serum hormones in CLL patients.

Hormone		*Mean ± SD*		
		CLL	*Control*	*p*
ACTH (ng/ml)		32.50 ± 23.89	38.10 ± 45.76	0.371
C (nmol/l)		780.70 ± 472.20	425.60 ± 179.08	0.000
FSH (nmol/l)	M	22.37 ± 13.24	2.47 ± 1.06	0.000
	W	44.09 ± 26.84	35.83 ± 14.39	0.273
LH (nmol/l)	M	17.48 ± 9.89	3.23 ± 1.24	0.000
	W	32.14 ± 22.72	35.56 ± 14.49	0.596
T (nmol/l)	M	14.93 ± 12.56	19.20 ± 9.12	0.216
	W	2.50 ± 4.19	1.94 ± 0.70	0.620
E (pmol/l)	M	185.87 ± 124.07	108.38 ± 39.13	0.020
	W	199.35 ± 188.86	227.16 ± 107.15	0.601
E/T	M	17.62 ± 9.14	8.86 ± 6.8	0.003
	W	115.65 ± 121.11	131.29 ± 81.08	0.650

Table 4 Serum hormones in early stage CLL.

Hormone		*Mean ± SD*		
		CLL I UTr	*Control*	*p*
ACTH (ng/ml)		33.36 ± 30.98	38.09 ± 45.76	0.608
C (nmol/l)		860.19 ± 473.82	425.59 ± 179.08	0.000
FSH (nmol/l)	M	16.72 ± 17.94	2.44 ± 1.06	0.004
	W	54.36 ± 40.26	35.83 ± 14.39	0.114
LH (nmol/l)	M	14.62 ± 13.52	3.23 ± 1.24	0.002
	W	39.94 ± 5.09	35.56 ± 14.50	0.669
T (nmol/l)	M	15.71 ± 7.56	19.20 ± 9.12	0.184
	W	1.94 ± 0.83	1.94 ± 0.70	0.988
E (pmol/l)	M	200.21 ± 133.68	108.38 ± 39.13	0.013
	W	172.97 ± 141.49	227.16 ± 107.15	0.258
E/T	M	17.76 ± 12.20	8.87 ± 6.09	0.040
	W	101.27 ± 101.99	131.29 ± 81.08	0.390

DISCUSSION

The present findings showing that PHA and Con A induced lymphocyte stimulation is reduced in CLL patients demonstrated that the functional capacity of the T lymphocyte, the primary immunocompetent cell, is altered in CLL. This is in agreement with many previous studies[16-19]. Interestingly lymphocyte proliferation to mitogens in CLL is not influenced by the clinical stage of the disease and markedly depressed cellular immunity has already been demonstrated in the early stages of CLL. Similarly, Platsoucas *et al.*[5] and Mittenham *et al.*[20] have not noted any correlation between abnormalities in the T cell population and the clinical staging system defined according to Rai[11]. The underlying mechanism for disordered T cell function in CLL is still unknown. However, defective CLL T cell proliferation has been related to at least 3 factors: defective CD3 membrane antigen expression, decreased endogenous interleukin 2 (IL2) production and suboptimal response to exogenous IL2[21,22].

Until now possible hormonal influences on the immune system in CLL have not been taken seriously into consideration. Our findings provide some evidence for some hormonal changes in CLL.

Table 5 Serum hormones in different stages of CLL.

Hormone		*Mean ± SD*		
		CLL I	*CLL II*	*CLL III*
ACTH (ng/ml)		33.17 ± 21.42	29.52 ± 19.37	35.49 ± 35.40
C (nmol/l)		935.45 ± 480.56	691.89 ± 456.68	724.63 ± 452.32
			p = 0.018	
FSH (nmol/l)	M	18.83 ± 21.39	21.38 ± 8.36	27.47 ± 8.99
			p = 0.02	
	W	44.77 ± 17.73	38.63 ± 20.06	47.96 ± 37.82
LH (nmol/l)	M	17.74 ± 16.35	16.97 ± 7.07	18.14 ± 6.01
	W	32.27 ± 14.92	29.96 ± 23.35	33.81 ± 28.97
T (nmol/l)	M	16.15 ± 8.78	17.28 ± 16.59	9.51 ± 3.94
				p = 0.05
	W	3.91 ± 7.03	1.81 ± 0.93	1.76 ± 0.62
E (pmol/l)	M	242.85 ± 156.66	178.75 ± 107.52	133.98 ± 46.31
				p = 0.005
	W	330.78 ± 255.11	172.19 ± 98.38	91.89 ± 45.89
				p = 0.005
E/T	M	21.30 ± 18.00	13.76 ± 8.51	19.72 ± 29.90
			p = 0.05	
	W	173.82 ± 164.70	112.40 ± 92.00	60.35 ± 51.15

Table 6 Serum hormones in stage I CLL treated (Tr) and untreated (UTr) patients.

Hormone		*Mean ± SD*		
		CLL I Tr	*CLL I UTr*	*p*
ACTH (ng/ml)		26.67 ± 19.06	36.57 ± 22.19	0.199
C (nmol/l)		856.60 ± 550.94	977.90 ± 446.50	0.454
FSH (nmol/l)	M	22.10 ± 1.41	18.39 ± 22.83	0.826
	W	42.24 ± 13.48	49.83 ± 24.96	0.408
LH (nmol/l)	M	23.75 ± 0.64	16.93 ± 17.31	0.596
	W	31.17 ± 16.06	34.50 ± 13.43	0.669
T (nmol/l)	M	29.50 ± 0.71	14.67 ± 7.93	0.019
	W	4.82 ± 8.28	1.72 ± 0.52	0.425
E (pmol/l)	M	391.00 ± 4.24	226.39 ± 156.72	0.164
	W	339.68 ± 293.39	309.39 ± 151.149	0.832
E/T	M	13.00 ± 0.60	22.22 ± 18.84	0.508
	W	163.42 ± 181.39	198.80 ± 130.08	0.700

Table 7 Serum hormones in stage II CLL treated (Tr) and untreated (UTr) patients.

Hormone		*Mean ± SD*		
		CLL Tr	*Control*	*p*
ACTH (ng/ml)		33.73 ± 18.95	12.21 ± 8.76	0.004
C (nmol/l)		725.55 ± 471.26	593.73 ± 414.19	0.394
FSH (nmol/l)	M	24.27 ± 6.46	12.71 ± 7.59	0.000
	W	40.42 ± 19.59	31.47 ± 3.30	0.509
LH (nmol/l)	M	19.15 ± 6.49	10.45 ± 4.28	0.001
	W	28.24 ± 22.75	36.83 ± 29.69	0.588
T (nmol/l)	M	16.37 ± 18.95	19.89 ± 6.40	0.614
	W	1.57 ± 0.52	2.77 ± 1.70	0.041
E (pmol/l)	M	189.91 ± 118.05	149.45 ± 66.83	0.375
	W	169.73 ± 101.17	182.00 ± 106.16	0.855
E/T	M	15.76 ± 8.79	8.50 ± 5.15	0.037
	W	121.08 ± 100.08	77.67 ± 38.80	0.485

Table 8 Serum hormones in stages III (III + IV) CLL in treated (Tr) and untreated (UTr) patients.

Hormone		*Mean ± SD*		
		CLL (III Tr)	*CLL (III UTr)*	*p*
ACTH (ng/ml)		32.27 ± 18.21	42.90 ± 49.76	0.371
C (nmol/l)		667.22 ± 418.84	873.90 ± 523.90	0.224
FSH (nmol/l)	M	29.15 ± 8.27	19.07 ± 9.05	0.075
	W	35.17 ± 16.47	68.04 ± 53.19	0.07
LH (nmol l)	M	18.95 ± 6.14	14.13 ± 3.67	0.215
	W	26.79 ± 12.45	44.84 ± 43.44	0.207
T (nmol/l)	M	9.25 ± 4.18	10.83 ± 2.55	0.541
	W	1.76 ± 0.79	1.74 ± 0.18	0.947
E (pmol/l)	M	125.07 ± 78.70	178.53 ± 92.73	0.310
	W	106.06 ± 56.06	71.64 ± 13.83	0.136
E/T	M	20.53 ± 32.80	15.67 ± 6.03	0.806
	W	73.40 ± 63.99	41.71 ± 12.10	0.219

Significantly increased cortisol levels in CLL patients may have serious effect(s) on cellular immunity. The association of higher cortisol levels and decreased lymphocyte mitogen reactivity is consistent with a number of studies reporting suppression of immune process by corticosteroids[23]. By an unknown mechanism, helper T cell function is preferentially affected, while suppressor T cell function remains intact[24]. As glucocorticoid effects on different classes of lymphocytes and various immunologic reactions are diverse[25–27], their physiological role in influencing immune responses under varying conditions may not simply be in the direction of global suppression. Almost any kind of threat to homeostasis or stress will cause plasma glucocorticoid levels to rise, and

increased plasma cortisol levels have been detected in the past during the course of immune responses[28,29]. Del Rey *et al.*[30] have shown that fluctuations in endogenous levels of blood glucocorticoids are relevant the the continuous endocrine surveillance of the immune cell network.

One hormonal complex which exerts profound influences on the immune system is the hypothalamic-pituitary-adrenal (HPA) cortical axis[8,9], the final product of which are glucocorticoids. The latter are crucial mediators of endocrine-immune interactions. Cortisol exerts a negative feedback control on the secretion of peptides from the HPA and the immune system. The HPA axis is activated by immunological challenge by tumor cells for example. The interactions between the immune system and the HPA axis are extremely complex, and, as yet, the role of these interactions is not fully understood. It is conceivable that by impeding a cumulative excessive expansion of lymphoid and accessory cells, this circuit (HPA) plays a role in preventing lymphoproliferative diseases. Imbalances in the immune-HPA axis may be involved in disease state. Interestingly both during the early phase of tumor growth and the immune response to antigens an increase in cortisol occurred. As far as our results are concerned, the highest levels of cortisol were already reached in the early stages of CLL. It is also notable that ACTH concentration is not down-regulated by high cortisol levels in CLL patients. It seems evident that there is dysregulation at the level of the HPA axis or the HPA-immune circuit, which may be involved in the pathogenesis of CLL. As demonstrated by Blalock *et al.*[31] ACTH can also be produced by stimulated lymphocytes[32]. Those findings strongly suggest the presence of an immuno-adrenal axis in which lymphocytes serve as a sensory function for stimuli such as tumor cells. We cannot exclude the participation or interference of this axis in the lymphoproliferation observed in CLL. ACTH also has a potential immunoregulatory function such as suppression of the antibody response by blocking T helper cell signals[33].

The data arising from the studies presented here indicate that dysbalance of sex hormones is present specially in male CLL patients. Higher levels of FSH, LH and E have also been detected in early stages of CLL. When female CLL patients were compared with the control group in respect to sex hormones, no significant changes were found. It is known that women with CLL have better prognosis than men with the same disease[34,35]. Thus we can only hypothesize that the imbalance of sex hormones in male CLL patients, because of the interference they cause in immune regulation, may in part be responsible for these differences. Accumulating data suggest that sex hormones exert a marked effect on events involved in the immune response[36], and T lymphocytes seem to be the primary target for sex hormone action. Sex hormones markedly modulate T cell mediated delayed-type hypersensitivity reactions[37–39], and may suppress the T lymphocyte proliferation stimulated in cultures by mitogens[36,40,41]. Studies in humans revealed that estrogen receptors (ER) are present on OKT-8 positive cells which have suppressor-cytotoxic functions[42]. In this respect the results of studies concerning the estrogen receptor status of CLL patients are discrepant: Danel *et al.*[43] and Zaniboni[44] have demonstrated significant ER activity in CLL patients, whereas Melo *et al.*[45] suggested that ER are rarely expressed on lymphocytes from patients with regular CLL. Thus it is difficult to conclude that ER expression plays any role in the pathogenesis of CLL. Returning to the T suppressor cells, it has been demonstrated, in vitro, that estrogens inhibit or deplete their functional activity[46] while male sex hormones maintain these functions. Another mechanism of the sex hormone action is the modulation of T lymphokines, IL2, and γ-interferon. Sex hormones modulate IL2 activity, and androgens, for example, maintain this activity[47,48]. It is not known precisely how sex hormones affect IL2 production, nevertheless manipulation of IL2 levels by sex hormones can profoundly alter immuno-competence. Sex hormones, for example, may influence the qualitative functions of lymphocytes by direct action on the DNA of the lymphocytes, and autoradiographic studies have revealed that[3] H-testosterone is incorporated into the nucleus of human lymphocytes[49].

Although estrogens and androgens have been reported to be both immunoinhibitory and immunostimulatory, mainly they suppress the cell-mediated immunity. However, it is possible that estrogens and androgens do not necessarily suppress the same lymphocyte populations. It is more plausible to suggest that the estrogen to androgen ratio may determine whether the circulating hormones will be immunostimulatory or immunoinhibitory[50]. In the case of CLL male patients only the E to T ratio is significantly increased, perhaps with a corresponding effect on immune reactivity and indirectly through this on the pathogenesis of disease.

In summary, we conclude from our data that: (1) CLL patients exhibit profound deficiency of cellular

immunity, which is not significantly influenced by the clinical stage of the disease or the specific treatment; (2) Defects in the immunoregulatory circuit integrated at the level of the hypothalamo-pituitary-adrenal cortical axis are present in CLL as demonstrated by the recorded values of ACTH and cortisol; (3) In male CLL patients significantly higher FSH, LH, E concentrations and an elevated E:T ratio are evident. These may possibly partly mediate the immune dysfunction.

These results are open to several interpretations. It seems evident from the above data that the abnormalities of the immune system observed in CLL may be connected with an imbalance of endocrine regulation. However, present knowledge about the interrelation of the immune and neuroendocrine systems is probably still very superficial. A better understanding of the immune and neuroendocrine circuits may provide new insights into the pathophysiology of CLL, lymphoproliferative diseases in general and perhaps other tumors as well. Such new information may well lead to many new diagnostic and therapeutic regimens, in the future.

Acknowledgements The author expresses her thanks to Drs. V. Hiir, J. Lehtmaa and M. Hein for their technical assistance.

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