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A Calf Thymus Acid Lysate Improves Clinical Symptoms and T-Cell Defects in the Early Stages of HIV Infection: Second Report

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Abstract—*Thymomodulin is a calf thymus acid lysate capable of inducing T lymphocyte maturation.*

Fifteen patients with HIV infection at different stages according to the Walter Reed classification were treated with 60 mg/*day of thymomodulin syrup for more than* 50 *days.*

Two WR6B subjects had clinical and immunological parameters unchanged and died, while the patient suffering from Kaposi's sarcoma presented an evident clinical and laboratory improvement with remission of the neoplasia.

The other 12 *patients ranging from WR2 to WR5B showed an improvement of clinical symptoms after thymomodulin therapy accompanied by the normalization of CD4/CD8 ratio* ($P < 0.001$). *This helpers/suppressors increase was due to a significant increase of CD4 cells* ($P < 0.01$) *and also to a decrease of the CD8 lymphocytes* ($P < 0.05$). *Thymomodulin administration did not cause an enhancement of the urinary levels of neopterin, a marker of T-cell activation.*

INTRODUCTION

ACQUIRED immunodeficiency syndrome (AIDS) is a pathological condition characterized by a severe immunity dysfunction including a total T lymphocytopenia and an elevation of serum immunoglobulins [1]. The etiological agent of AIDS is a retrovirus named human immunodeficiency virus (HIV) [2] with a selective tropism for CD4 cells [1, 3]. Some evidence shows that HIV needs CD4 cell activation for its replication [4–9].

The clinical features of patients with HIV infections, as recently underlined by the Walter Reed staging classification [10], can range from an asymptomatic condition (with viremia or antibody or both), through AIDS related complex (ARC) with chronic generalized lymphoadenopathy to true AIDS in which T-cell depletion is associated with various opportunistic infections. There are several therapeutic approaches toward HIV infection based on three strategies: treatment with drugs directed against the replication of the etiologic agent [11–13], the immunerestorative therapy with biological response modifiers [14–17] and the use of immunosuppressive agents with the aim of not offering the causative virus new target cells to damage [9]. All these therapeutic efforts, singly or as combinations, are currently under investigation.

In a preliminary study some of us used in a few AIDS and ARC patients a calf thymic derivative (thymomodulin) which improved clinical symptoms and partially corrected the T-cell defects [18]. In the present work we report that the treatment with thymomodulin induced a clinical improvement and a statistically significant increase of CD4 lymphocytes and of CD4/CD8 ratio and a decrease of CD8 cells in the early stages of HIV infection.

MATERIALS AND METHODS

Fifteen HIV infected patients (11 males, 4 females; mean age: 28.8 ± 6.6) were treated for more than 50 days with 60 mg/day of thymomodulin syrup (for the preparation of this thymic derivative see Ref. [19]). Two patients (Nos. 1 and 3) had experienced several treatments without benefit.

The following parameters were evaluated:

—clinical symptoms;

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Table 1. T-Cell subsets and neopterin serum levels in HIV infected subjects before and after treatment

Case	Risk group	Stage*	Before thymomodulin CD4/CD8 ratio	CD3†	CD4†	CD8†	Neopt (nmol/l)	After thymomodulin CD4/CD8 ratio	CD3†	CD4†	CD8†	Neopt (nmol/l)
1	Homo-sexual	WR6B	0.61	487	176	285	7.5	0.73	848	330	450	4.8
2	Hetero-sexual	WR4K	0.07	314	19	260	—	0.80	626	222	412	—
3	Homo-sexual	WR6B	0.39	726	184	470	7.2	0.45	444	115	257	5.4
4	Homo-sexual	WR2B	0.64	882	321	545	6.4	1.52	1378	711	666	4.8
5	Homo-sexual	WR3B	0.58	1009	401	606	5.6	1.08	1289	835	378	4.9
6	IV drug user	WR5B	0.93	1266	550	587	6.9	1.52	1285	697	459	10.7
7	Homo-sexual	WR2B	0.33	1534	429	1268	8.5	1.02	1394	922	900	8.6
8	IV drug user	WR5B	2.04	1280	840	420	7.7	1.66	1275	835	521	4.1
9	IV drug user	WR2B	0.89	1270	560	622	5.3	1.51	1262	710	473	9.4
10	IV drug user	WR2B	0.40	1680	451	1103	1.2	1.12	1365	841	750	6.1
11	IV drug user	WR5B	0.73	851	368	491	8.7	2.00	878	533	266	5.6
12	IV drug user	WR5B	1.00	913	359	359	11.5	1.57	1221	861	548	3.9
13	Homo-sexual	WR2	0.92	2023	720	776	4.0	1.72	1419	897	522	2.3
14	Homo-sexual	WR3B	1.53	1086	434	282	4.8	2.13	1138	535	250	8.8
15	IV drug user	WR5B	1.78	1470	735	411	4.9	1.76	1040	457	260	3.2

*According to Walter Reed Staging classification.
†Cells/mmc.

—delayed hypersensitivity;
—peripheral lymphocyte count;
—T-cells and T-cell subsets: enumerated in indirect immunofluorescence after density centrifugation and labelling with monoclonal antibodies (OKT3, OKT4 and OKT8, Ortho Diagnostics, Raritan, NJ, U.S.A.) and their number expressed as cells/mm;
—neopterin serum levels (nmol/l): as described by Hausen *et al.* [20].

On the basis of clinical and laboratory data all the patients were classified according to the Walter Reed staging classification [10]: the characteristics of study subjects are reported in Table 1.

The statistical analysis of the results was performed with Student's t test for paired data and P values less than 0.05 were considered statistically significant.

RESULTS

The results are summarized in Tables 1–3. The two WR6B patients (cases 1 and 3) had clinical and immunological parameters unchanged and died a little while after the end of the study. On the contrary the patient (case 2) with Kaposi's sarcoma presented an evident clinical and laboratory improvement with remission of the neoplasia.

The other 12 patients ranging from WR2 to WR5B, all showed the resolution of the fever after thymomodulin therapy; in six patients lymphoadenopathy disappeared and in only one did thrush continue (Table 2). These cases exhibited a statistically significant enhancement of CD4/CD8 ratio from 0.98 ± 0.53 to 1.55 ± 0.34 (Table 3). This increase was due to a significant increase of the absolute number of CD4 lymphocytes and also to a decrease of the CD8 cells (Table 3). The absolute

Table 2. Clinical symptoms in HIV-infected subjects before and after treatment

Case	Risk group	Before thymomodulin				After thymomodulin			
		Lap	Fev	Thr	OI	Lap	Fev	Thr	OI
1	Homosexual	+	–	–	+	+	–	–	+
2	Heterosexual	Kaposi's sarcoma				No relapses			
3	Homosexual	+	–	–	+	+	–	–	+
4	Homosexual	+	+	–	–	–	–	–	–
5	Homosexual	+	+	–	–	–	–	–	–
6	IV drug user	+	+	+	–	+	–	–	–
7	Homosexual	+	+	–	–	–	–	–	–
8	IV drug user	+	+	+	–	–	–	–	–
9	IV drug user	+	+	–	–	–	–	–	–
10	IV drug user	+	+	+	–	+	–	–	–
11	IV drug user	+	+	+	–	+	–	–	–
12	IV drug user	+	+	+	–	+	–	+	–
13	Homosexual	+	+	–	–	–	–	–	–
14	Homosexual	+	+	–	–	+	–	–	–
15	IV drug user	+	+	+	–	+	–	–	–

Lap = chronic lymphoadenopathy; Fev = fever; Thr = thrush; OI = opportunistic infections.

Table 3. T-Cell subsets and neopterin serum mean levels in 15 HIV-infected subjects before and after treatment

	Before thymomodulin	After thymomodulin	*P* level
CD3*: (cells/mmc)	1272 ± 356	1245 ± 158	n.s.
CD4* (cells/mmc)	513 ± 169	736 ± 156	<0.01
CD8* (cells/mmc)	622 ± 296	499 ± 201	<0.05
CD4/CD8* ratio	0.98 ± 0.53	1.55 ± 0.34	<0.001
Neopterin* (nmol/l)	6.29 ± 2.60	6.03 ± 2.70	n.s.

*Mean ± standard deviation.

number of CD3 lymphocytes and the serum neopterin levels did not show statistically significant changes.

No side-effects were observed during the treatment with thymomodulin.

DISCUSSION

There is evidence that the thymus is a target organ in AIDS patients and the hypothesis that HIV could penetrate into the gland is not uncommon [21]. In fact, autopsy findings reveal a marked involution of the thymic gland with depletion of lymphocytes and epithelial cells, reduction and calcification of Hassall's corpuscles [22–24]. A recent immunohistological study detected a partial loss of the differentiation antigens of the thymic epithelium in which the FTS (Facteur Thymique Serique) content is decreased [25].

The epithelial damage could account for the decline of the thymic endocrine function in these patients [26–29]. The defective T-cell differentiation [30] is corrected *in vitro* by cultured human thymic epithelial monolayer cells [31]. These data seem to justify the therapeutic approach of this condition with thymic hormones [14, 15, 29, 32, 33].

Thymomodulin is an orally administrable calf thymic acid lysate, which has been demonstrated to be capable of inducing the maturation of pre-T (null) cells [34, 35]. There are clinical reports that this drug restores FTS (Facteur Thymique Serique) activity in elderly humans [36] and exerts an immunomodulating action during infectious diseases [19, 37]. Thymomodulin treatment in healthy volunteers does not increase urinary levels of neopterin (personal data not shown), a pyrazino-pyrimidine compound considered a new biochemical marker for clinical assessment of T lymphocyte activation [38, 39].

On the basis of this knowledge we decided to treat a group of HIV-infected patients with this thymic derivative. While the clear improvement

observed in the WR4K patient could be due to coincidence, the results obtained in 12 patients ranging from WR2 to WR5B suggests that the early use of thymomodulin during HIV infection can be useful in the treatment of such condition. In fact after 50 days of therapy with this drug we noticed a clinical improvement supported by a statistically significant increase of T-helper cells and of the T-helper/T-suppressor cell ratio, with a decrease of T-suppressor lymphocytes, which seem to play an important role in the pathogenesis of AIDS [40].

These results confirm that in patients with full-blown AIDS thymic derivatives have transient or no effectiveness on the clinical and immunological status [14, 29], while they can increase CD4 cell number [14, 32, 33] and T-cell functions [15] in pre-AIDS patients.

Some authors [41, 42] reported very high serum neopterin levels in HIV-infected subjects, others only in symptomatic patients [43]. In the present work the mean of the serum values of this marker were only slightly increased before therapy with thymomodulin and this thymic derivative did not change this parameter. This could mean that thymomodulin exerts an immunomodulating effect on the T-cells without promoting their activation. In this study, as in others with thymic hormones [15, 33], no development of frank AIDS was observed in thymomodulin treated patients but a long follow-up period is necessary.

A double blind clinical trial is now under way to confirm these encouraging results and to exclude the interference due to a possible change of life habits in these patients.

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