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Preliminary observations using HIV-specific transfer factor in AIDS

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Abstract

Twenty five HIV-1-infected patients, at various stages (CDC II, III and IV) were treated orally with HIV-1-specific transfer factor (TF) for periods varying from 60 to 1870 days. All patients were receiving antiviral treatments in association with TF. The number of lymphocytes, CD4 and CD8 subsets were followed and showed no statistically significant variations. In 11/25 patients the number of lymphocytes increased, whilst in 11/25 decreased; similarly an increase of the CD4 lymphocytes was observed in 11/25 patients and of the CD8 lymphocytes in 15/25. Clinical improvement or a stabilized clinical condition was noticed in 20/25 patients, whilst a deterioration was seen in 5/25. In 12/14 anergic patients, daily TF administration restored delayed type hypersensitivity to recall antigens within 60 days. These preliminary observations suggest that oral HIV-specific TF administration, in association with antiviral drugs, is well tolerated and seems beneficial to AIDS patients, thus warranting further investigation.

Abbreviations: c.equ.: cell equivalent; CMI: cell-mediated immunity; D: day; DTH: delayed type hypersensitivity; KS: Kaposi's sarcoma; LMT: leucocyte migration test; PHA: phytohaemagglutinin; TF: transfer factor.

Introduction

Transfer factor (TF) has been efficaciously used for treating various viral pathologies [1–8], and several years ago preliminary observations suggested that it might have a beneficial effect in HIV-infected patients [9–10].

In a preliminary attempt to assess its clinical activity and confirm the absence of adverse side effects in AIDS patients, anti-HIV TF was produced following standard methods, i.e., by animal immunization and subsequent replication in tissue culture, and was orally administered to HIV-infected patients at various stages of the disease. Because of the constraints concerning AIDS clinical trials and the difficulties in funding a coordinated multicentric clinical study, as well as in recruiting AIDS patients, due to the fact that most patients are included in existing standard antiviral protocols which do not allow the adjunct of additional therapeutic agents, the present data have been collected by several clinicians over an extended time period, on an open trial basis.

Treatment was administered over variable time periods, the aim being for each clinician to establish that TF is compatible with conventional anti-HIV treatments and does not produce undesired side-effects, whilst it may induce beneficial clinical or laboratory reactions. In most cases, the studies were discontinued after a few months, and to this day, only 4 patients have received this therapy for more than 2 years.

However sketchy and anecdotal these observationsmay seem, they are suggestive of the role that TF may play in AIDS immunotherapy, which, it seems now, should be started as early as feasible, i.e., in

seropositive patients, and continued uninterrupted for as long as possible. This TF-based immunotherapy can be associated with antivirals.

Materials and methods

Transfer factor

Six to eight week old BALB/c mice received one SQ injection of 2×10^9 viral particles of HIV-1 (strain HTLV-IIIB) [11], and simultaneously one SQ injection of 10^6 HIV-1-infected LDV/7 cells [12]. The animals were sacrificed 21–25 days after immunization, and lymphocytes were obtained from their spleens after lysing the red blood cells by a hypotonic shock. The lymphocytes were subsequently lysed by sonication and filtered through two millipore membranes having respectively cut-off points of 1000 and 10000 Daltons. The cell dialysate was used for the induction of the LDV/7 cell line as previously described [13]. Induced cultures were grown to a total of 10^{10} cells and then harvested. They were subsequently lysed by sonication and filtered through 1000 and 10000 Dalton cut-off filters. The dialysates were freeze-dried and kept at -20°C. The HIV-1 activity of each batch was tested in the leucocyte migration test (LMT) [14,15] using formalin fixed [16] HIV-infected LDV/7 cells. The freeze-dried dialysate was mixed with lactose and encapsulated at 5×10^7 cell equivalent (c.equ.) per capsule. It was administered at an average dose of 3 capsules per week.

Patients. Patients were in stages II, III and IV, following CDC's classification (Table 1). Nineteen were males and 6 females. They received TF for variable time periods. Most initial studies were planned as phase-I clinical trials. However, when results started to be clinically encouraging, it was occasionally decided by the treating physician to continue the TF treatment for longer periods. When possible, the following parameters were assayed before/during and, in certain patients, after TF administration: WBC, total lymphocytes, platelets, CD4, CD8, NK, β2-microglobuline and p24 antigenaemia. Skin tests were carried out in 14 patients using the multitest Mérieux.

Results

Table 1 shows variations of 3 parameters: number of total lymphocyte and CD4, CD8 subsets in 23 patients from the day of TF administration (D0) up to D270. An overall decrease is observed at D30 in all three parameters, followed by a slight increase and stabilization of the CD4 and CD8 numbers. At the end of the observation period, the total number of lymphocytes and CD4 cells remains slightly lower, whilst the number of CD8 lymphocytes is slightly higher. The differences are not statistically significant, nor were statistically significant differences found in the evolution of the other laboratory parameters assayed. The clinical condition improved or remained stable in 20/25 patients, whilst in 5/25 a deterioration was noticed.

Some patients received TF for long periods (Tables 2–4). Patient PB1 (stage IVD)(Table 2) started AZT treatment in 1988 and TF treatment in 1990, which continued, with occasional interruptions, to this date. At the onset of TF administration he was suffering from HIV encephalitis, and survival prognosis did not exceed 6 months. For nearly 3 years the patient's follow up was irregular and was carried out by physicians not participating in the study. On his own initiative, the patient used to discontinue all treatments for short (1–3 months) periods. Nonetheless, since 1993 his follow-up has become more regular and he received, together with TF, combinations of AZT, DDI and 3TC. In the last 3 years a marked decrease of the total lymphocytes number and that of the 2 lymphocyte subsets was noticed. Albeit this deterioration, the patient's clinical condition has remained relatively stable over the last 5 years; he gained weight (3 kg) and has maintained normal professional activity. Herpes bouts and Kaposi's sarcoma (KS) lesions, present since 1988, were managed by conventional treatments.

The treatment of patient PB2 (stage IIB) (Table 3), sexual partner of patient PB1, followed a similar pattern of interruptions. However, the improvement from D1020, when his follow up became regular, is evident. Not only his clinical condition improved and KS lesions remained stable, but five years after the onset of TF treatment, administered in association with AZT, the number of lymphocytes, CD4, CD8 and NK cells have increased.

Table 4 shows the long term evolution of patient N.18 of Table 1. An improvement of laboratory parameters was seen soon after the onset of the TF treatment, which was added to the antivirals (AZT and DDI) the patient was receiving the 2 preceding years. The clinical condition showed dramatic improvement; fatigue and depression subsided, and the patient resumed a very active professional life. Dermal KS lesions remained stable, whilst a lung KS lesion regressed

Table 1. Patients

Patients No.	CDC Sex	stage	DO			D30			D60			D90			D180			D270			Clinical Response
			LYM.	CD4	CD8	LYM.	CD4	CD8	LYM.	CD4	CD8	LYM.	CD4	CD8	LYM.	CD4	CD8	LYM.	CD4	CD8	
1	F	IVD	1511	230	951	1106	135	723	1096	132	715	917	140	601	1030	110	555	1094	120	534	1
2	F	IVC2	2054	611	923	1591	455	754	1550	421	731	1473	430	729	1620	415	691	1504	394	715	1
3	F	II	1131	160	731	1607	190	1065	1583	205	1076	1677	230	1095	1620	190	997	1532	212	1031	2
4	M	IVD	2245	92	1534	2614	103	1754	2195	112	1449	1862	121	1501	1790	115	1401	2014	106	1328	2/3
5	M	IVD	1930	256	1078	2228	330	1223	2176	335	1235	2073	350	1189	1815	406	1179	1911	30	1255	1/2
6	M	IVC2	1242	78	577	1301	180	615	1503	200	818	1881	185	910	1670	205	989	1644	818	1149	1/2
7	M	IVC2	1454	107	819	1360	145	753	NA	NA	NA	752	165	470	862	160	421	NA	152	1015	1/2
8	M	IVC2	989	301	481	993	276	435	1299	292	516	NA	NA	NA	1398	282	830				1/2
9	M	IVC2	1083	131	601	1485	142	815	1765	145	871	NA	NA	NA	1495	NA	715				2/3
10	M	II	2129	364	1136	1846	359	951	1928	NA	1134	2090	277	1284	2031	299	1191				2
11	M	IVC2	2709	709	1603	1235	513	596	1347	491	999	2173	NA	NA	1784	439	1061	1821	426	1076	1/2
12	M	IVD	2016	752	980	1860	783	817	2240	912	839	NA	NA	NA	2035	1051	993				2
13	M	IVC2	3264	1134	1128	3478	1189	1003	3478	1199	1108	2394	1129	1089	2215	1039	1108	2167	992	1096	2
14	M	IVD	2700	98	989	3864	183	1118	3087	125	1097	3186	302	1287	NA	NA	NA				1/2
15	M	IVD	667	73	353	NA	NA	NA	NA	NA	NA	672	73	295	1030	92	442	1400	126	644	1
16	M	IVD	440	4	202	NA	NA	NA	608	6	310	285	2	133	500	5	260	410	4	197	3
17	F	II	1000	140	560	NA	NA	NA	1188	154	819	1383	179	912	1360	122	992	759	60	561	2
18	M	IVD	589	35	385	655	45	412	NA	NA	NA	442	17	229	426	17	238	398	4	87	3
19	M	IVD	460	4	317	311	3	174	700	1	257	500	1	248	800	2	325	615	2	351	2
20	M	II	1218	329	NA	NA	NA	NA	NA	NA	NA	1300	468	637	NA	NA	NA				1
21	F	IVD	400	88	148	NA	NA	NA	NA	NA	NA	630	170	246	NA	NA	NA				1
22	M	IVD	1100	11	710	931	9	475	672	7	372	NA	NA	NA	NA	NA	NA				3
23	F	IVD	1320	317	779	NA	NA	NA	1566	454	783	NA	NA	NA	NA	NA	NA				1
Mean			1462	261	772	1674	296	804	1671	315	851	1363	293	737	1379	243	787	1328	246	788	
SD			797	287	291	936	305	370	783	320	337	693	329	425	523	255	363	615	310	402	
Nb			23	23	23	17	17	17	18	17	18	18	17	17	17	16	17	13	14	14	

Lymphocytes, CD4 and CD8 counts of 23 patients during TF intake. The number, sex and CDC stage for each patient are shown in the first 3 columns. Patients' age: 24–54, with the exception of patient N.23, age 6. 14 patients were followed for at least 270 days, and 9 for 180 days; the mean values with the standard deviation (SD) are shown at the bottom of each column. Clinical response is summarized as: 1 = improvement, 2 = stable condition, 3 clinical deterioration. N = patient's number; NA = not available; Nb = number of samples.

Table 2. Patient PB1 (Stage IVD)

Post TF Day	LYM.	CD4	CD8	NK	p24	TREAT.
60(Oct 1990)	1269	178	520	ND	170	AZT
1020	713	71	377	7	NA	"
1140	864	120	449	0	1000	DDI
1170	360	75	129	8	720	"
1210	756	90	347	8	620	"
1270	683	88	321	7	260	"
1300	527	42	315	6	510	"
1360	400	25	120	0	610	"
1390	400	46	199	0	540	"
1450	504	31	233	5	1300	"
1540	600	36	247	0	1250	"
1630	493	19	305	5	1550	"
1720	500	30	340	5	1275	AZT
1810	900	63	558	45	600	AZT+3TC
1870	400	20	200	8	720	3TC

Patients' laboratory data between D60 and D1020 are not available; clinical condition remained stable throughout treatment, despite multiple KS lesions present since 1988 and HIV encephalitis since 1989. Lymphocytes (LYM), CD4 and CD8 or natural killer cells numbers are shown; p24 = values of HIV p24 antigenaemia in μg; TREAT = asociate anti-HIV treatment.

Table 3. Patient PB2 (Stage IIB)

Post TF Day	LYM.	CD4	CD8	NK	p24	TREAT
60(Oct 1990)	1197	203	515	ND	0.0	AZT
1020	1428	228	899	186	ND	"
1140	2065	309	1321	165	0.0	"
1170	1800	342	1134	90	0.0	"
1210	1972	276	1321	256	0.0	"
1270	1950	312	1306	253	0.01	"
1300	1987	298	1352	219	0.0	"
1360	1676	329	856	101	0.0	"
1390	1922	274	914	173	0.0	"
1450	1890	273	844	208	0.20	"
1540	1927	289	1310	212	0.25	"
1630	1674	267	1104	201	0.0	"
1720	1800	234	1332	234	0.0	"
1810	1800	306	1206	216	0.0	"
1870	2000	300	1380	280	0.0	"

Lymphocytes (LYM), CD4 and CD8 or natural killer cells numbers are shown; p24 = values of HIV p24 antigenaemia in μg; TREAT = associated anti-HIV treatment.

without specific therapy as did voluminous plantar warts. Nearly three years after the onset of the TF treatment, the CD8 cell number shows a slight decrease, whilst the total lymphocyte number and the CD4 subset show an increase. Table 5 shows laboratory data of patient N.16 of Table 1. Despite regular TF administration this patient failed to respond. His clinical condition showed a deterioration, parallel to his haematological parameters.

In an attempt to confirm the role of TF in restoring delayed type hypersensitivity (DTH), skin tests were carried out in 14 anergic patients (stage IVC2) using the multitest Mérieux. They were tested 30 and 60 days after initiation of daily oral TF administration (Table

Table 4. Patient N.15 (Stage IVD)

DAY	LYM.	CD4	CD8	NK	p24
0	667	73	353	0	0.04
90	672	73	295	0	0.0
120	900	81	423	0	0.0
180	1030	92	442	0	0.0
210	537	80	225	0	0.0
240	1400	126	644	14	0.0
270	888	106	390	0	0.0
330	1000	80	530	0	0.0
360	811	56	364	0	0.0
420	1044	43	282	11	0.10
450	800	43	279	8	0.80
480	1300	68	353	0	0.40
580	1118	89	570	11	NA
660	727	65	341	22	0.40
750	534	64	186	5	0.50
1030	1000	90	340	10	2.80

Patient N.15 (stage IVD) suffered from pulmonary KS. Since post TF D360 all anti-KS chemotherapy was discontinued; KS remained stable and on D750 a partial regression was noticed. Lymphocytes (LYM), CD4 and CD8 or natural killer cells numbers are shown; NA = not available; p24 = values of HIV p24 antigenaemia in μg.

Table 5. Patient N16 (Stage IVD)

DAY	LYM.	CD4	CD8	NK	p24	TREAT
0	440	4	202	9	15	AZT
60	608	6	310	12	40	"
90	285	2	133	3	24	"
120	588	5	252	5	55	"
180	500	5	260	5	100	"
240	500	5	395	10	110	"
270	410	4	197	8	90	"
300	561	5	353	11	60	"
360	597	5	368	12	120	"
420	467	1	151	10	190	"
450	262	2	146	2	85	"
480	328	NA	NA	NA	NA	"
540	455	3	205	9	800	"
580	498	4	278	20	NA	"
660	200	2	112	2	1560	"
750	100	1	45	NA	1700	"

Lymphocytes (LYM), CD4 and CD8 or natural killer cells numbers are shown; p24 = values of HIV p24 antigenaemia in μg; TREAT = associated anti-HIV treatment.

6). 8/14 and 10/14 were found positive respectively at D30 and D60.

Table 6. Skin-test converion following TF administration

Day:	0	30	60
Number of patients with positive skin tests:	0/14	8/14*	10/14**

A group of 14 anergic AIDS patients (stage IVC2) received orally a daily dose of 5.10^7 cell equ. for 60 days. 8/14 at D30 and 10/14 (71%) at D60 showed a restored skin test response to the multitest Mérieux. *P=0.01; **P=0.0005 Fisher's exact tet was used for computing the statistical significance.

Discussion

Transfer factor was used in AIDS patients as early as 1986 [9,10]. In their study, Carey and coworkers reported that they were able to restore DTH, as assessed by skin tests, in previously anergic AIDS patients, and also to increase their in vitro blastogenic response to phytohaemagglutinin (PHA) and antigens [10]. However, the improvement in the immune response diminished after the TF injections were discontinued.

These observations are consistent with ours shown on Table 6: orally administered TF was capable of restoring skin test reactivity to recall antigens within 30–60 days of the initiation of treatment. Although these studies were not pursued, and DTH was not systematically assayed in all patients, they appear to be of interest. Indeed, they confirm observations made by Gottlieb et al. [17,18], suggesting that a 6 month treatment using IMREG^R, an immunomodulator contained in the TF dialysate, can restore DTH in ARC patients and also retard disease progression. This effect seems to be independent of TF's antigenic specificity. Moreover, a correlation between cutaneous DTH response and survival prognosis is now established, as is the in vitro IL-2 production following antigen or PHA stimulation [19]. Thus, from these data one may now surmise that TF treatment can delay disease progression, and this can be predicted by monitoring the patients' aforementioned CMI parameters.

An improvement in survival can be inferred in the present study by the clinical improvement noticed in 14/25 patients, and the stable condition of 6/25. It is worth observing that the improvement of the clinical condition is not always reflected in the assayed laboratory parameters, suggesting that the latter do not always provide an accurate picture of the clinical situation. Be that as it may, it seems pertinent to collate the clinical results of this study to those mentioned by Ortega-Fernandez et al. [20]: in a 4 year controlled trial in asymptomatic HIV-infected patients, the authors

observed a significant difference in disease progression between the TF treated and the placebo group; only 3/43 (7%) of the TF receiving patients developed AIDS, whilst 27/78 (28%) AIDS cases were recorded in the control group.

The same authors report inhibition of HIV replication by dialysable leucocyte extracts obtained from pooled leucocytes of healthy volunteers. Although the mechanism of the phenomenon is not elucidated and its extrapolation to an in vivo situation, considering the concentrations involved, seems prima facie improbable, these observations corroborate the contention that the TF dialysate, as a result of the numerous moieties contained therein, is a multifacet activity product. Thus, in some pathological conditions, *unspecific* TF can, at least partially, restore the immune functions and achieve clinical improvement. Specificity, nonetheless, is of the essence. In this context, we report elsewhere the absence of an effect of HIV-specific TF on herpes relapses, whilst HSV-specific TF, subsequently administered to the same patients, proved efficacious in controlling the herpes bouts [21].

The evolution of the HIV infection is not predictable at the individual level; thus, several years may elapse before a seropositive patient progresses into disease, and it is now quasi-certain that not all HIV-infected patients will. Similarly, survival varies from one patient to another. These individual variations should have provided leads for the pathogenesis of the syndrome and the underlying mechanisms of resistance, as it was suggested by one of us in 1987 [22]. This has not been the case. Too confident, because of the swift advances of virology in identifying the virus, the main research effort was concentrated in comprehending its functions, in view of producing antiviral compounds capable of inhibiting its mechanisms of replication, whilst the second goal has been the preparation of a vaccine capable not only to protect against infection, but also against disease progression. In this targeted, fast moving research, implemented by the latest techniques of molecular biology, the main problem was gradually lost from sight: the syndrome itself with its CMI implications and, consequently, the patient.

However, it has eventually become obvious that *natural* mechanisms to resist the HIV infection are present, and have permitted several seropositives to escape disease, as are immune mechanisms allowing them to resist infection. Thus, although the focus of attention to CMI was long in coming, several indications were pointing out that cellular immunity was playing a crucial role in the syndrome [23–25], and not only because one subset of its effector cells was the target of the virus. This has been discussed to some extent elsewhere [26]; suffice to report here some salient evidence. Borrow et al. [27] have shown that viraemia of symptomatic HIV-1 patients was controlled by CTL recognising gp160, an envelope glycoprotein of the virus, and the level of the HIV-specific CTL activity paralleled the efficiency of control of primary viraemia. Thus, patients with strong cytotoxic responses showed rapid reduction of acute plasma viraemia and antigenaemia, whilst, contrariwise, both viraemia and antigenaemia were poorly controlled in patients with low gp160-specific cytotoxic activity. Rowland Jones et al. [28] have reported that certain Gambian prostitutes, who remained uninfected (both PCR- and sero-negative) despite multiple unprotected sexual intercourse, presented HIV-specific CTL lymphocytes. This observation not only implies that CMI plays a key role in AIDS, but also suggests that it can prevent infection.

Contrasting to the failure of humoral immunity to control the virus, because of its high mutation rate, CMI seems able to overcome this aspect. Indeed, the sexual partners of the Gambian prostitutes offer a vast array of viral strains without succeeding in breaking the immune resistance of the recipient. Thus, the contention - discussed elsewhere [26] - that specific TF might be used as a prophylactic vaccine against HIV infection finds support in clinical and laboratory data. However, the prophylactic use of TF is not novel. In a thorough clinical trial, Steele et al. have shown that VZV-specific TF can protect immunocompromised leukaemic children from varicella zoster infections [1], whilst Viza et al., using HSV-specific TF, protected mice against HSV lethal challenge [5].

The data reported here are consistent with clinical results obtained with specific TF in treating other viral infections. When they are collated with the data reported in recent years on the role of the CMI in controlling the HIV infection, they make the investigation of the use of HIV-specific TF for the management, and even the prevention of AIDS, urgent and compelling.

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