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Orally administered HSV-specific transfer factor (TF) prevents genital or labial herpes relapses

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Abstract

Forty-four patients suffering from genital (22) and labial (22) herpes were orally treated with HSV-1/2-specific transfer factor(TF). TF was obtained by in vitro replication of a HSV-1/2-specific bovine dialysable lymphocyte extract. Treatment was administered bi-weekly the first 2 weeks, and then weekly for 6 months, most patients received 2–3 courses. The total observation period for all patients before treatment was 26660 days, with 544 relapses, and a relapse index of 61.2, whereas the cumulative observation period during and after treatment was 16945 days, with a total of 121 relapsing episodes and a cumulative RI of 21.4 ($P<0.0001$). Results were equally significant when the 2 groups of patients (labial and genital) were considered separately. These observations confirm previous results obtained with bovine HSV-specific TF, and warrant further studies to establish HSV-specific TF as a choice of treatment for preventing herpes recurrences.

Abbreviations: c.equ. : Cell equivalent, CMI : Cell mediated immunity; HIV : Human immunodeficiency virus; HSV : Herpes simplex virus; LMT : Leucocyte migration test; LST : Lymphocyte stimulation test; MI : Migration index; RI : relapse index; TF : Transfer factor.

Introduction

The prevention of recurrences in genital and/or labial herpes remains an unresolved problem for the clinician. Indeed, although the acute infection can be treated with interferons and antiviral drugs inhibiting viral replication, the prevention of relapses is far more difficult to obtain, no drug can eliminate the 'quiescent' virus. However, cell mediated immunity (CMI) appears to play a crucial role in preventing the 're-emergence' of the virus [1–4], an increase of the immune response can reduce the frequency and the severity of relapses [2,4].

In order to prevent manifestations of the HSV infection, an increase of the immune response can be attained through: a) specific Transfer Factor (TF) [2,5–10] or b)vaccines [1,11–15]. Vaccines, heretofore, have produced uncertain and unconvincing results. In contrast, it has already been shown that HSV-specific TF can prevent herpetic relapses [2,5–9].

In the present study we have investigated the clinical efficacy of a bovine lymphocytes dialysate, used in previous studies [2,5,7,8], after its in-vitro-replication in large standardized batches [16,17].

Materials and methods

Patients. Forty-four patients, 22 suffering of genital, and 22 of labial recurrent herpetic infections (24 female, 20 male; age 16–79) entered the study. Two were AIDS patients (stage IVD and IVC1), the others did not suffer from any additional pathology. All had at least a year long history of herpes relapses with 2–27

episodes per year. Most patients, prior to TF administration, were treated during relapses with topical applications of α-interferon and/or acyclovir. Criteria for recurrence was the presence of at least one papular, vesicular, or ulcerative lesion. Prodromal symptoms e.g. tingling, itching or pain in the genital or facial areas, not accompanied by visible lesions, were considered as prodromal episodes, but not recorded as relapses.

Transfer factor. Bovine transfer factor was produced and its activity assessed as previously described [2,5,7,8]. Briefly, calves were injected with HSV-1 and HSV-2 live viruses, and sacrificed 3–4 weeks later after skin testing had produced evidence for reactivity to HSV. The activity of the TF obtained from the calves' lymphocytes was assessed in vitro, using the LMT, and in vivo, using a protection-to-lethal-HSV-challenge mouse model [10]. The active bovine TF, thus obtained, was replicated in tissue culture using standard methods described elsewhere [16,17]. The in vitro replicated TF was encapsulated and orally administered at an average dose of 4×10^8 cell equivalent (c.equ.) per week in the first 2 weeks of treatment (induction phase) and then at 10^8 c.equ. per week for the following 6–12 months.

Immunological assays. ELISA was used for the evaluation of anti-HSV antibody titers in the patients' serum (Behring-Germany). The LMT was carried out as described by Centifanto et al. [3] and by Sϕberg et al. [18], whereas the LST has been described elsewhere [19]. HSV antigens used for studies other then ELISA, were obtained from Ismunit (Pomezia, Italy).

Results

Immunological studies. Table 1 shows the LMT results obtained in 15 patients studied before, and in 9 during and after TF treatment. In this sample, only 20% of the patients were positive to HSV antigens, albeit the presence of anti-HSV antibodies in the serum of all of them. In contrast, 3 previously unreactive patients, showed significant reactivity after TF treatment, with a mean migration index (MI) ranging from 0.68 to 0.81.

Table 2 shows results obtained with the LST. The ability of the patients' lymphocytes to incorporate tritiated thymidine, in the presence of HSV antigens, is significantly increased during and after TF treatment. Wilcoxon's test for paired data shows statistical significance both at low (10^{-1})(P=0.05) and high (10^{-4})(P<0.0001) dilutions of the antigen.

Table 1. LMT in presence of HSV antigens.

	N	Mean MI	N of positive patients
before treatment			
LMT 1:25	15	0.97±0.08	3/15
LMT 1:50	15	1.00±0.04	
LMT 1:100	15	1.01±0.04	
after TF treatment			
LMT 1:25	9	0.68±0.30	
LMT 1:50	9	0.78±0.24	6/9
LMT 1:100	9	0.81±0.22	

N = number of patients tested; Mean MI = mean migration index (positive if <0.85). The ratios 1:25 to 1:100 represent the dilution of the antigen with reference to the standard solution (The starting solution is defined as 1/8 of the dilution used for complement fixation).

Table 2. LST in presence of HSV antigens.

	Before treatment (N:12)				
Ag. Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Control
mean $\log_{10}$ dpm	2.97	3.35	3.13	2.99	2.96
SD	0.53	0.66	0.72	0.35	0.41
	After treatment (N:5)				
mean $\log_{10}$ dpm	3.78	3.86	3.82	4.01	3.28
SD	0.99	0.80	0.73	0.33	0.29
P	0.05	NS	NS	0.0001	NS

N = number of patients tested. Ag. Dilutions (10^{-1} to 10^{-4}) represent the dilutions from the original solution of the antigen used in the LST. (The original solution is defined as 1/8 of the dilution used for complement fixation.) Mean $\log_{10}$ dpm = the average dpm values of triplicate cultures of all the patients studied. SD = Standard deviation. Wilcoxon's test for paired data was used for the statistical evaluation.

Clinical results. The number of patients, days of TF treatment and follow-up, and relapse indices (RI) are reported in Table 3. Twenty-two patients suffering from labial and 22 from genital herpes have been treated with HSV-specific TF for, respectively, 7605 and 7768 days. The number of relapses observed before treatment in both groups was 544, with a cumulative RI of 61.2; during and after treatment the number of relapses dropped to 121 and the RI to 21.4 (P<0.0001). The cumulative RI of labial herpes was reduced 5 fold when compared to the period preceding the TF administration (from 68.7 to 12.9), whilst that of genital herpes dropped from 56.3 to 28.2 (P<0.0001 in both cases).

Two patients of our cohort suffered from AIDS. Patient GZ (Stage IVD), (table 4) and patient MC (Stage IVC1) (table 5) experienced 4 recurrences in the 10 months proceeding TF treatment and had an

Table 3. Relapses before and during/after TF treatment

	All patients (44)		Labial (22)		Genital (22)	
	Before	After	Before	After	Before	After
Follow-up(d)	26660	16945	10526	7889	16134	9056
Treatment (d)		15373		7605		7768
Mean treat (d)	349±195		346±217		353±175	
Min. treat.(d)		71		71		117
Max. treat. (d)		961		961		715
N Relapses	544	121	241	34	303	87
Cumulative RI	61.2	21.4	68.7	12.9	56.3	28.2
	P<0.0001		P<0.0001		P<0.0001	

N Relapses = number of relapses; (d) = days; Min. Treat., Max. Treat. = Minimum & maximum duration (days) of individual treatments; RI = 100 × number relapses ÷ Months of follow-up. Mean treat. = Mean duration of treatment for all patients. Wilcoxon's test for paired data was used for the statistical evaluation.

Table 4. AIDS Patient GZ

Treatment:		Days	relapses	RI
Zovirax	+ HIV-TF	300	4	40
Zovirax	+ HIV-TF + HSV1/2-TF	300	0	0

AIDS (stage IVD) Patient GZ, (age 48) was suffering from labial and genital herpes and was also receiving chemotherapy for Kaposi's sarcoma. This patient experienced 4 relapses in the 10 months before HSV-specific TF treatment, despite the administration of HIV-specific TF and acyclovir, with an individual RI of 40; he didn't notice any new bout after starting specific HSV-1/2 TF treatment.

Table 5. AIDS Patient GP

Treatment:		Days	relapses	RI
Zovirax	+ HIV-TF	338	4	36
Zovirax	+ HIV-TF + HSV1/2-TF	366	0	0

AIDS (stage IVC1) Patient MC, (age 32) was suffering from recurrent oesophageal herpetic lesions. The mean duration of bouts was 12 days. Treatment with HSV-specific TF suppressed these recurrences; he only noticed abortive (prodromal) symptoms.

individual RI of 40 and 36. After the beginning of HSV-1/2-specific TF treatment, no further relapses occurred.

Discussion

Despite years of intensive research, relapsing herpes still remains an unresolved clinical problem. If antivirals, such as acyclovir, are a powerful weapon against acute infections, the clinician remains unarmed when facing a chronic situation with regular recurrences. Indeed, acyclovir is usually effective but only while administered; bouts resume as soon as the treatment is interrupted. Furthermore, in addition to the cost and the obligation to the patient of a daily pill-ingestion, the absence of long-term toxicity of this therapy has not been established, and the risk of selecting resisting strains is always present. Contrariwise, TF has, in other studies, proved its efficacy in treating not only HSV, but also other viral infections, without any side effects or drawbacks for the patients.

The present data confirm previous clinical observations and also laboratory results, suggesting that herpes patients' CMI fails to react to HSV antigens [2–5,9], despite the presence of anti-HSV antibodies in their serum. Some studies suggest that the CMI defect in these patients is not only confined to HSV antigens [20]. Furthermore, several reports have established that cytotoxicity, viz. cytotoxic T lymphocytes (CTL), play an important role in controlling herpes infections [21–24]. Thus, it is plausible that patients suffering from herpes relapses may have impaired cytotoxic reactions to HSV-infected cells. Recently obtained data offer a mechanism for the impaired CMI reactions: HSV interferes with immune surveillance by expressing a cytoplasmic protein, ICP47, which operates a retention in

the endoplasmic reticulum of newly synthesized class I MHC molecules, allowing the infected cells to escape recognition and lysis [25,26].

Thus, the rationale for attempting to manufacture herpes vaccines seems today mistaken. But, despite continuous failures, the race has not subsided in the last two decades [11–14,27–39]. Nevertheless, the rationale seemed equally erroneous fifteen years ago. Indeed, herpes patients always appeared to lack CMI reactivity to HSV antigens, despite a chronic infection by the HSV and a normal humoral response. Additional immunization of such patients with vaccines has a low probability to do what 'natural immunization' by the live-virus failed to achieve: live, weakened viruses have always been considered better immunizers than inactivated preparations [40]. Therefore, it is not surprising that what remains today from the past attempts are forlorn hopes, raised by excessive publicity and hyped press releases, by those with vested interests in such enterprises. Be that as it may, even if a vaccine showing some efficacy were ever discovered, vaccination will still present drawbacks, e.g. patients obligation to return to hospitals for recall injections. It should be emphasized once again here, that TF has never produced any adverse side effects, even when it was used for long-term treatments, i.e., for several years, and in injectable form [41,42].

The rationale for using adoptive immunisation, i.e., specific TF, in herpes is simple: control a viral infection due to CMI impairment, by specifically boosting the CMI defences to the virus. Theoretically, TF should be able to overcome an immune impairment be it genetic, as, for instance, in the Wiskott-Aldrich syndrome, or virus-induced, as seems to be the case in herpes infections, and it does. The first observations, suggesting that HSV-specific TF can be clinically efficient, date back to 1983 [5,6]. They have, since, repeatedly been confirmed [2,7–10], and the present data are an additional confirmation of our contention that oral administration of HSV-specific TF produces a clinical improvement, by increasing the patient's CMI response to HSV antigens. In the LST, the improvement appears statistically significant, both at the highest (1/10000) and lowest (1/10) antigen dilutions (respectively $P<0.0001$ and $P<0.05$). Studies, now in progress, should determine the most appropriate test (LMT or LST), and antigen dilution, for predicting a favourable clinical response. So far, our observations suggest that an increased reactivity, at the lowest concentration of the HSV antigen in LST, seems to correlate with a decrease of the clinical manifestations.

It is of interest to mention here, preliminary observations showing that TF administration increased the presence of soluble HLA class I antigens in 4 genital herpes patients studied to this effect. Similar findings were observed in 36 patients suffering from recurrent herpes keratitis who were treated with TF [43]. The underlying mechanism of this finding is not fully understood. One may speculate that TF, by increasing HSV-infected cell lysis and/or by decreasing the HLA molecules intracellular entrapment, increases circulating HLA. Also, it is known that certain cytokines, i.e., gamma–interferon and IL-4, produced during the immune response by T cells, enhance the expression of MHC class I molecules [44]. By increasing cytokine production, TF may also increase HLA class I expression. Recently, it has been shown that HLA molecules, released from cultured cells, can selectively be adsorbed by syngeneic or allogeneic lymphoid cells, and that MHC class I proteins, produced by recombinant DNA technology, block T cell stimulation by competing with membrane MHC molecules [45]. An observation suggesting that these molecules may contribute to the regulation of the interactions between activated cytotoxic T cells and their targets. Be that as it may, and although little is known of the role of soluble HLA class I antigens, their levels (in the serum and on the cell surface) could probably be utilized as a surrogate marker to follow immune activity, as in the case of AIDS [46]. However, the significance may be different for each physiopathological condition.

Despite a strongly impaired immune system, due to the advanced stage of their disease, the 2 AIDS patients with herpes relapses responded to HSV-specific TF. These data warrant further investigation, not only because HSV infections are a frequent problem in AIDS patients, but also in view of the recent hypothesis of a possible synergy between HSV-1 and HIV [47]. But, this observation offers an additional argument for TF's specificity. Indeed, prior to their treatment with HSV-specific TF, the AIDS patients were receiving HIV-specific TF, together with acyclovir, which had no effect on their herpes relapses. The recent identification of a herpes virus associated with Kaposi's sarcoma [48], makes the claims that specific TF can be successfully used against herpes virus infections, be HSV, EBV [43] or CMV [49], more interesting, since one may speculate that a specific TF against the KS virus would be as efficient as TF prepared against other viruses of the herpes family.

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