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Transfer Factor for the Treatment of HBsAg-Positive Chronic Active Hepatitis (42033)

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Abstract. Transfer factor was obtained from four patients having recovered from acute type-B viral hepatitis. It was replicated *in vitro* using the LDV/7 lymphoblastoid cell line. This *in vitro*-produced transfer factor specific for hepatitis B (TFdL-H) was administered to 10 randomly selected patients with biochemically and histologically proven HBsAg-positive chronic active hepatitis (CAH) at 15-day intervals over a 6-month period. In three out of four initially HBeAg-positive patients, anti-HBe antibodies appeared when the HBeAg disappeared. In one of these patients and in two other HBsAg-positive patients, the appearance of anti-HBs antibodies was noted. The improvement in several biochemical parameters of the TFdL-H patients was statistically significant when compared with those of another group of 10 randomly selected untreated CAH patients. Liver biopsies in six out of eight treated patients showed a histological improvement at the end of the treatment. These results suggest that TFdL-H may be used with beneficial effect for the treatment of HBsAg-positive CAH.

Since it has been suggested that HBsAg-positive chronic active hepatitis (CAH) is the result of a defective immune response to the hepatitis B virus (HBV) (1–4), adoptive immunotherapy using transfer factor (TFd) extracted from lymphocyte pools of convalescent B or non-B acute viral hepatitis (AVH) patients has been attempted with varying results (5–7). The two main problems with TFd extracted from peripheral blood lymphocytes from various donors at different times are the inconsistency of its biological activity and the limited amounts of active material obtained. *In vitro* production of unlimited amounts of dialyzable transfer factor (TFdL), with standardized biological activity against various antigens, using lymphoblastoid cell lines, can overcome these problems and has been described extensively elsewhere (8–12).

We report here the results of a pilot study conducted on 10 patients with HBsAg-positive CAH treated with TFdL specific for antigens of HBV origin (TFdL-H). This TFdL-H was obtained by inducing the LDV/7 cell line (13) with a TFd from patients who had recovered from a HBsAg-positive AVH.

Patients, Materials, and Methods. *Patients.* Twenty patients admitted to the department entered the study. The criteria for inclusion were histological diagnosis of CAH by the usual methods (14), abnormal transaminases, and stable HBs antigenemia for 12 months. None of the patients had previously received steroids or was a drug addict. The sex, age, and HBV serum markers of each patient before TFdL treatment are shown in Table I. Ten of the patients were randomly selected to receive TFdL-H.

Double blind histological evaluation of liver biopsies was performed immediately before and 1 month after the end of the treatment in 8 out of the 10 TFdL-H-treated patients. In addition to the physical examination, transaminase, HBsAg, HBeAg, anti-HBs, anti-HBc, and anti-HBe (AUS-RIA II, AUSAB, CORAB, ABBOTT/HBe, Abbott Laboratories) serum levels were assessed every 2 weeks. Total bilirubin, serum albumin, serum gamma globulin, gamma-GT, and prothrombin activity were evaluated monthly.

The 10 remaining HBsAg-positive CAH patients were used as controls. These patients did not receive any treatment during the observation period. They had a follow-up identical to that of the TFdL-H patients. However, for ethical reasons, the liver biopsy for the control group was not performed at 6 months.

Transfer factor: (a) Production. Transfer factor was produced *in vitro* using the LDV/7 lymphoblastoid cell line. The replication

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TABLE I. SEX, AGE, AND HBV SERUM MARKERS IN BOTH TFdL-H AND CONTROL GROUP PATIENTS AT THE BEGINNING OF THE STUDY

Patient	Sex	Age (years)	HBsAg	HBeAg	Anti-HBs	Anti-HBc	Anti-HBe
				TFdL-H group			
G.G	M	38	+	+	−	+	−
A.S.	M	51	+	−	−	+	+
Z.S.	F	29	+	+	−	+	−
R.S.	M	42	+	−	−	+	+
B.S.	M	47	+	+	−	+	−
C.F.	M	37	+	+	−	+	−
G.D.	M	27	+	−	−	+	+
S.M.	M	18	+	−	−	+	+
F.D.	F	25	+	−	−	+	+
M.D.	M	23	+	−	−	+	+
				Control group			
G.S.	F	48	+	−	−	+	+
A.M.	M	37	+	+	−	+	−
L.M.	F	33	+	+	−	+	−
R.R.	M	29	+	+	−	+	−
A.N.	F	43	+	−	−	+	+
E.G.	F	52	+	−	−	+	+
D.D.	M	44	+	−	−	+	+
D.B.	M	44	+	−	−	+	+
S.R.	M	24	+	−	−	+	+
M.F.	M	26	+	−	−	+	+

procedure has been described elsewhere (8–11). Briefly, TFd from 5×10^7 pooled peripheral blood lymphocytes taken from four anti-HBs-positive patients, 3 months after their recovery from a HBsAg-positive AVH, was used to induce 5×10^7 LDV/7 cells cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The inducing TFd was not removed from the culture but fresh medium was added until a large number of cells was reached (approx 4×10^{10}). The approximate time required for the culture to reach this number of cells was 3 weeks. The cells were then harvested and the TFdL-H was extracted in the usual manner (15), aliquoted, and stored lyophilized.

(b) Test of activity. The TFdL-H was biologically characterized by its ability to transfer *in vitro* the reactivity to HBsAg in the leukocyte migration inhibition test–microdroplet technique (16). Briefly, 5×10^6 lymphocytes from an apparently healthy HBsAg-negative and anti-HBs-negative donor were incubated 24 hr at a 1:5 ratio (i.e., 2.5×10^7 LDV/7 cell equivalent was added to 5.10^6 fresh lymphocytes) with crude TFdL-H or its Sephadex G-25 fractions in 5 ml of RPMI 1640 + 20% of noninactivated FCS at 37°C in an atmosphere of 5% CO_2. After three washings, the cells were incubated for 2 hr with or without 1 μg/ml of purified HBsAg or 400 μg/ml of a chemically defined antigen: mono-(*p*-azobenzenearsonate)-*N*-chloracetyl-L-tyrosine conjugated with bovine albumin (ABA-BSA). At the end of the incubation, they were washed twice and aliquots of 2×10^5 cells were reincubated in 0.2 ml of RPMI 1640 complemented with 1% pooled AB serum for an additional 24 hr in 0.3-ml conical plastic tubes. The supernatants from this incubation were collected and assayed at dilutions ranging from 10^{-1} to 10^{-5} for the presence of migration inhibition activity on allogeneic human polynuclear cells. Briefly, agar microdroplets (2 μl) containing 2×10^5 polynuclear cells were incubated for 18 hr with the various supernatant dilutions. The migration of the cells was evaluated by projection with an inverted light microscope and measurement, using a "V" scale, of the outer circle (migration of the cells) and of the inner circle (agar microdroplet). The latter was

then subtracted from the former. Each dilution was tested in quadruplicate. Statistical analysis of the data was performed using Student's t test, matching mean migration areas in the presence of TFdL-H against migration areas in the presence of TFdL-H and antigen. TFdL-D61 and its Sephadex G-25 fractions were used as control. TFdL-D61 is a replication product originating from patients with transitional cell carcinoma of the bladder (TCCB) and was strongly reactive against bladder tumor cells when tested in the leukocyte migration technique (9, 10).

(c) TFdL administration. Each patient was injected im every 2 weeks for 6 months with TFdL-H extracted from 2×10^8 lymphoblastoid cells resuspended in 5 ml of pyrogen-free saline. After a 3-month treatment-free interval, patient Z.S. received TFdL-H for an additional 7 months.

Results. *In vitro studies.* The crude TFdL-H was unable to induce lymphocyte inhibition factor (LIF) in nonsensitized lymphocytes *in vitro.*

Since previous experience with TFdL against tumor antigens had shown that a batch of TFdL inactive *in vitro* may be able to transfer reactivity when injected *in vivo,* TFdL-H was fractionated on a Sephadex G-25 column. Two of the fractions thus obtained were found to be able to induce LIF against HBsAg *in vitro* in two out of three experiments, with significant inhibition of migration varying from 22% ($P < 0.05$) to 31% ($P < 0.001$). However, no reactivity was transferred to ABA-BSA in any of the three experiments. Similarly, no transfer of reactivity for HBsAg or ABA-BSA was observed with a different TFdL (D61), or its G-25 fractions, thus confirming the specificity of the TFdL-H for HBsAg.

Clinical efficacy. The parameters used for evaluation are described in Ref. (14). TFdL-H-treated patients suffering from asthenia, anorexia, and malaise showed a clear clinical improvement at the end of the treatment with the disappearance of these symptoms and body-weight gain. Furthermore, there was a significant decrease in transaminase serum levels after the first 3 months of TFdL-H therapy (SGPT $P < 0.01$ and SGOT $P < 0.005$) in all patients. Seven out of ten patients reached normal values for SGPT by the end of the treatment (Fig. 1).

A significant improvement was also observed for the other biochemical parameters tested (Table II and Fig. 2).

Double blind histological evaluation was

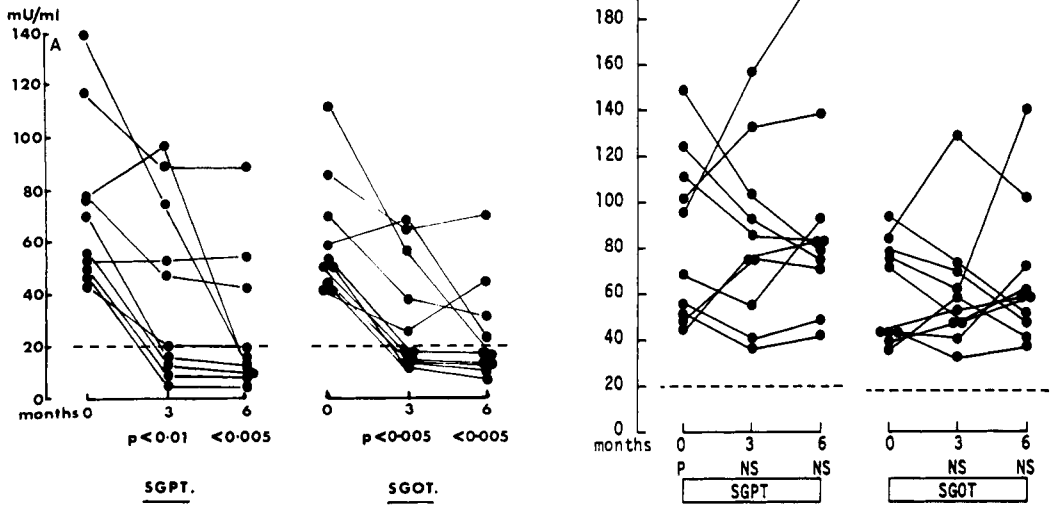

FIG. 1. (A) SGOT and SGPT levels in the TFdL-H group of patients over 6 months of treatment. (B) SGOT and SGPT levels in the control group.

TABLE II. BIOCHEMICAL CHANGES IN TFdL-H-TREATED PATIENTS AND CONTROLS DURING THE 6-MONTH PERIOD OF OBSERVATION

	SGOT (mU/ml)	SGPT (mU/ml)	Total bilirubin (mg/dl)	Gamma-GT (mU/ml)	Serum albumin (g/dl)	Serum gamma globulin (g/dl)	Prothrombin activity (%)
				TFdL-H group			
mean	−36.6	−48.2	−0.32	−59.0	0.34	−0.20	11.6
SD	25.3	34.3	0.26	116.9	0.62	0.26	9.4
				Control group			
mean	7.3	6.3	−0.02	13.6	0.10	0.20	−2.6
SD	32.5	51.2	0.16	38.9	0.43	0.25	6.7
P	<0.01	<0.05	<0.05	<0.05	NS	<0.01	<0.01

carried out on liver biopsies of eight patients after treatment. No changes were observed in two patients, whereas four patients showed a decrease in activity with reduction in piecemeal necrosis and disappearance of bridging necrosis; in one patient an aspect of chronic persistent hepatitis (CPH) was observed and in the eighth, disappearance of inflammation and necrosis, with portal and peri-portal fibrosis (Table III) was observed.

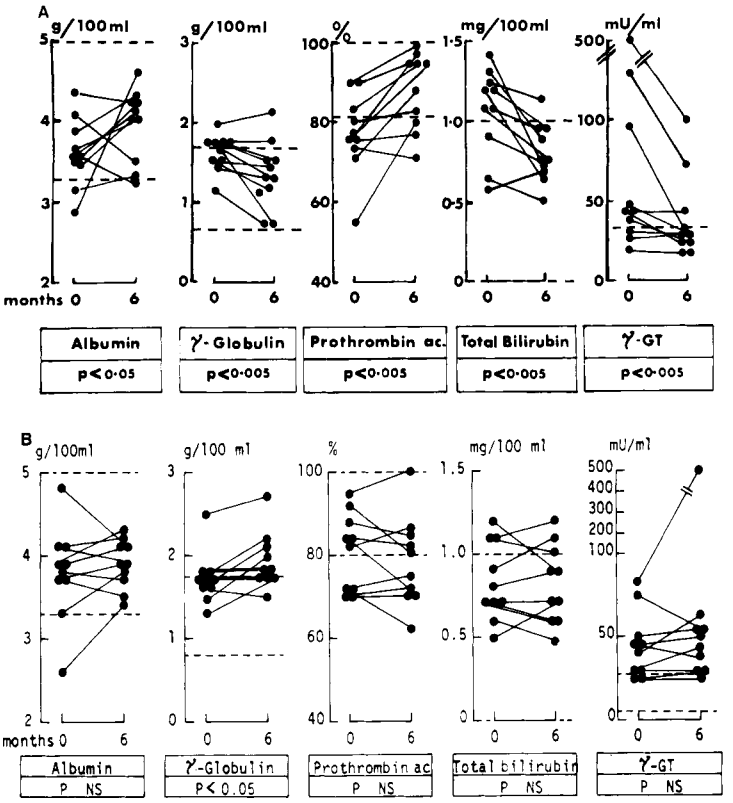

FIG. 2. (A) Serum albumin, serum gamma globulin, prothrombin activity, total bilirubin, and serum gamma-GT in the TFdL-H group of patients before and after treatment. (B) Serum albumin, serum gamma globulin, prothrombin activity, total bilirubin, and serum gamma-GT in the control group.

TABLE III. HISTOLOGICAL FINDINGS BEFORE AND AFTER TREATMENT IN THE TFdL-H GROUP OF PATIENTS

Patients	Before	After
G.C.	Severe CAH	Severe CAH
A.S.	Severe CAH	Severe CAH
Z.S.	Severe CAH	Moderate CAH
R.S.	Severe CAH	Moderate CAH
B.S.	Severe CAH	Moderate CAH
C.F.	Severe CAH	Moderate CAH
G.D.	Severe CAH	ND
S.M.	Moderate CAH	CPH
F.D.	Moderate CAH	PPF
M.D.	Moderate CAH	ND

Note. CAH: Chronic active hepatitis; CPH: chronic persistent hepatitis; PPF: portal and periportal fibrosis; ND: not done.

At the end of the 6-month treatment period a decrease, although not statistically significant, was observed in the HBsAg serum titer in all the TFdL-H-treated patients, whereas anti-HBs antibodies appeared in two patients. Moreover, in two out of the four initially HBeAg-positive patients, appearance of anti-HBe antibodies and complete disappearance of HBeAg occurred 5 months after the beginning of the TFdL-H therapy (Fig. 3).

Patient Z.S., treated for an additional 7 months, showed clinical and biochemical improvement only during this second period of treatment. All her altered biochemical parameters returned to normal values. Furthermore, decrease in HBsAg and anti-HBc serum titers (from 1/30,000 to 1/500 and from 1/10,000 to 1/500, respectively), the disappearance of HBeAg and the appearance of anti-HBe and anti-HBs antibodies were also observed. The liver biopsy in this patient at the end of the second treatment period revealed signs of CPH.

No side effects related to TFdL-H injections were observed, thus confirming our previous experience with other cancer patients (9, 10).

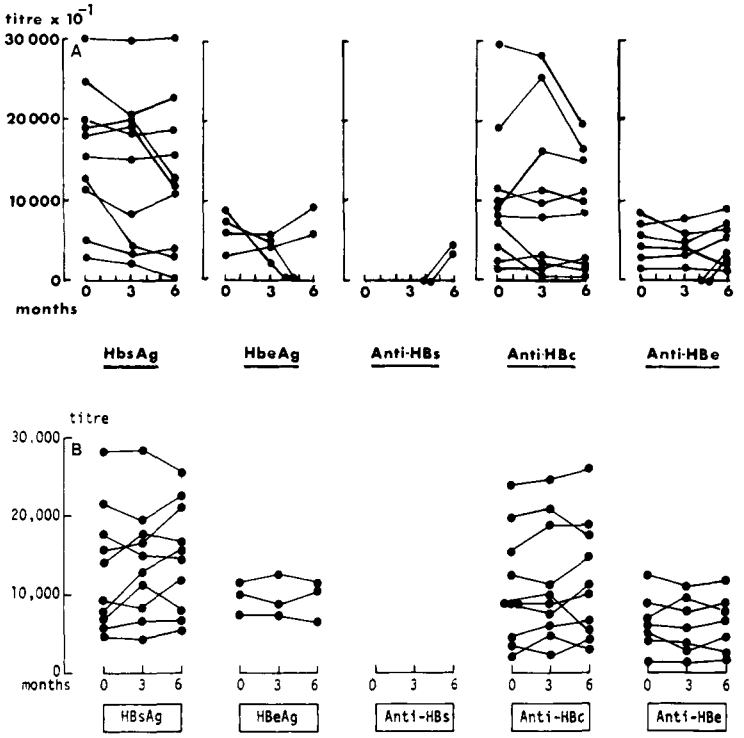

FIG. 3. (A) Variations in the titer of HBV serum markers in the TFdL-H group of patients during the 6-month period of treatment. These results include those of patient Z.S. during her first course of TFdL-H treatment. (B) Variations in the titer of HBV serum markers in the control group.

Biochemical analysis of the control group and statistical analysis of the comparison with the results of the TFdL-H group are given in Table II. At the beginning of the study a significant difference between the two groups of patients was found only for bilirubinemia which was higher in the treated group. However, 6 months later, serum gamma globulin levels had significantly increased in the control group, but no differences were observed for the other parameters. The statistical analysis of the results, using Wilcoxon's test for paired data, showed a significant improvement in transaminase, serum gamma globulin levels, prothrombin activity, and gamma-GT in the TFdL-H group as compared to control group values. No changes in HB serum markers were observed in the control group.

Discussion. There have already been attempts to treat CAH patients using transfer factor. Tong *et al.* (7) treated an HBsAg-positive CAH patient in remission, with TFd obtained from a patient recovered from a type-B AVH. At the end of the treatment a lymphocyte stimulation in the presence of HBsAg was observed, but no variation was noticed in other biochemical parameters (e.g., circulating HBsAg or anti-HBc titers).

Shulman *et al.* (6) carried out a double blind study on nine HBsAg-positive CAH patients. In five patients treated with specific TFd obtained from B and non-B hepatitis patients, a significant clinical and histological improvement was noticed in comparison to the four placebo-treated patients.

In two HBsAg-positive CAH patients, Jain *et al.* (5) administered a series of three TFd injections. The TFd for the first injection was obtained from lymphocytes of apparently healthy blood donors with no history of hepatitis. TFd for the second and the third injections was obtained from lymphocytes of patients who had recently recovered from HBsAg AVH. The third injection contained three times the quantity of the TFd of the second injection. Only a temporary increase in transaminases and a fall in serum complement after the second injection was noticed.

In the present trial, the patients showed a significant clinical improvement with correction of the biochemical parameters at the end of the TFdL-H administration; six out of eight patients in whom the liver biopsy was repeated after treatment presented a histological improvement. It thus seems that the treatment of HBsAg-positive CAH patients with specific TFdL-H produces a decrease in the activity of the disease with subsequent clinical improvement.

It is worth mentioning that although the control group did not receive transfer factor of different specificity during the time of the trial, 6 of the 10 patients in this group did receive nonspecific TFdL-D61 for 6 months at the end of the trial. No improvement during this period was observed.

The mechanism responsible for this effect is not clear. The lack of increase in the serum of the parameters indicating a liver cell necrosis, before improvement, does not confirm the hypothesis of Jain *et al.* (5) who suggest that the administration of specific TFd would determine immediate lysis of infected liver cells mediated by antibody and complement-dependent killer lymphocytes. It rather seems that TFdL-H acts on cell-mediated immunity by inducing a decrease in the effector cell function with subsequent decrease in liver cell damage. This suppressor activity has already been suggested by others (6, 17) and might be due to a stimulation of the T-suppressor lymphocytes. This would be consistent with the regulatory effect of TFd and the presence of specific suppressor factors (18, 19).

The appearance of anti-HBs antibodies in three patients and the decrease in the HBsAg titer in five seem to indicate that antibody production is stimulated in the TFdL-treated patients. Furthermore, sero-conversion in three out of four HBeAg-positive patients suggests that the TFdL treatment decreases viral production: HBeAg is indeed considered to be a serum marker of active viral replication (20, 21).

The improvement in patient Z.S., only after the second course of treatment, pleads in favor of a prolonged TFd treatment, as is the case in other pathological conditions associated with apparently moderate immune deficiencies (22).

The chromatography studies of the TFdL-H confirm that a preparation apparently

inactive *in vitro* can become active if the inhibitory fractions are removed. The *in vitro* screening of TFd preparations for activity is therefore advisable after filtration through Sephadex G-25 to remove inhibitory moieties. However, one should bear in mind that negative results can sometimes be obtained with active TFd as was the case here. It is thus advisable to screen TFd *in vitro* using several leukocyte donors.

The present results suggest that specific TFdL may be successfully employed in the treatment of HBsAg-positive CAH patients. It is of some interest to add here that encouraging results have recently been observed in the treatment of recurrent infections of herpes simplex of type 1 or 2, using bovine-specific transfer factor (12). It thus seems that specific transfer factor may be used for the treatment of chronic viral infections. Since the problems of large scale production of specific TFd have now been overcome, further controlled clinical trials in this area should be encouraged. Confirmation of the present observations and contentions could be of interest.

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