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REVIEW

# Transfer Factor in Virus-Associated Malignancies: An Underestimated Weapon in Prevention and Treatment of Cancer

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**Abstract:** Transfer factor (TF) is a lymphocyte extract of low molecular weight capable of transferring antigen-specific information to T-lymphocytes. A number of reports have documented its efficacy in the control of acute viral infections, particularly those associated with herpes viruses. There have been also several reports of clinical trials utilizing Epstein-Barr Virus-specific TF in associated malignancies, particularly Burkitt's lymphoma and nasopharyngeal carcinoma. One of the more important advances in the field has been the ability to produce standardized TF in tissue culture or by animal immunization, allowing the opportunity to develop well-controlled clinical trials, and the possibility to replicate them in independent studies. This review discusses the studies that have provided the best information regarding the efficacy of TF in fighting viral infections and suggests areas where clinical trials could be most useful, such as the prevention of human papilloma virus induced cervical cancer and human T-cell lymphotropic virus induced adult T-cell leukemia/lymphoma.

**Keywords:** viruses, transfer factor, cancer, prevention, treatment, herpes, EBV, hepatitis, HIV, immunity, T-lymphocytes

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## Introduction

Transfer factor (TF) is an immunomodulator of low molecular weight, described for the first time by Lawrence in the early 1950s and capable of transferring antigen-specific information to T-lymphocytes.[1]

A number of reports have documented its efficacy in the control of acute viral infections, particularly those associated with herpes viruses. There have been early reports of clinical trials utilizing TF in Epstein-Barr Virus (EBV)-associated malignancies, particularly Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). Since those reports, the number of malignancies clearly associated with oncogenic viruses,[2–6] as well as others believed to be virus-associated[7–10] has provided additional possibilities to test the effectiveness of TF. One of the more important advances in the field has been the ability to produce standardized TF in tissue culture or by animal immunization, allowing the opportunity to develop well-controlled clinical trials, and the possibility to replicate them in independent studies.[11]

In this review we assess the studies that have provided the best information regarding the efficacy of TF in fighting viral infections and suggest areas where clinical trials could be most useful.

In the first studies postulating the existence and establishing the concept of TF, Lawrence showed that Delayed Type Hypersensitivity (DTH) to a given antigen as assessed by skin tests could be transferred from one individual to another via cell-free extracts obtained from the leukocytes of an immunized donor. He assumed that this adoptive transfer of immunity was due to an unidentified moiety that he named Transfer Factor (TF) and he surmised that its molecular weight was less than 12,000 DA, as it filters through a standard dialysis bag. Its MW is now estimated ca. 5000 DA. Since that time, TF preparations for clinical and experimental studies have been obtained by disrupting immune lymphocytes, dialyzing the lysates and using the dialyzed material for in vitro tests or in vivo clinical or animal studies.[12]

TF should thus be defined as a dialysate obtained from the lymphocytes of an immune donor capable of transferring cell-mediated immunity (CMI) to a naïve human or animal recipient for a given antigenic specificity. Its activity can be assessed by skin tests in vivo, but also in vitro using assays such as the leukocyte migration inhibition (LMI),[13,14] lymphocyte stimulation (LS)[15,16] or cytotoxicity[17] tests.

Numerous reports have supported Lawrence's original observations and established that the dialyzable extracts thus obtained are capable of transferring antigen-specific immune information in vitro to naïve lymphocytes or in vivo to patients or experimental animals. There is no evidence that TF produces an antibody response; it only addresses cellular immunity. Since the early 1970s, TF has been used more often than not successfully for the treatment of viral, parasitic, and fungal infections, as well as an adjuvant treatment in autoimmune, allergic and malignant disorders, and there're over one thousand reports pertaining to in vitro, animal and clinical research.[12] The fact that CMI plays a crucial role in the control of infectious, parasitic, and autoimmune diseases, as well as cancer appears to account for its success.

The first report of cancer treatment by specific TF was that of an osteosarcoma patient by Fudenberg and his colleagues in 1976 who chose osteosarcoma because of the possibility that it had a viral etiology.[18] In the original publication, his group demonstrated an increase in cell-mediated cytotoxicity and other laboratory markers in patients given transfer factor prepared from the lymphocytes of family members whose lymphocytes had strong cytotoxicity against osteosarcoma cells.[17] A subsequent report suggested a prolonged disease free interval in patients treated with TF. In the follow-up to the original study, six patients with Stage I and II disease (locally confined or with only one small metastasis) as of 10–32 months after surgery had no demonstrable metastases by clinical, chemical, or radiologic criteria, whereas by 20 months approximately 80% of patients in the same stages of disease would have been expected to develop pulmonary metastases. In this study, two patients were maintained on "*non-specific*" TF and developed metastases and died.[19]

Since this report, a number of confirmatory studies have documented the success of transferring cellular immunity as demonstrated by a variety of in vitro assays, but clinical effectiveness has not been consistently observed. Indeed, until 1974, the only source of TF for treating patients was pooled leukocytes from blood donors or household contacts

when antigen specificity was desired. Obviously, this limited material supplies, and furthermore the biological potency and specific activity of the extract varied from one preparation to another and reproduction of the observations by different investigators was unpredictable. In fact, the precise antigenic specificity of the various batches of material used was not known, although presumably large, since each batch reflected the collective immune experience of several individuals. For this reason, these preparations were improperly called "*non-specific*," indicating multiple but unknown specificities. Thus, despite several encouraging and sometimes spectacular reports in the early 1970s, the clinical use of TF was curtailed by the dearth of material with standardized and consistent activity. This may explain the reason why some clinical observations were occasionally irreproducible and controversial. Indeed, if the treatment is ineffective, one may hypothesize that the required specificity was absent from the extract, or present in insufficient amounts, as studies have shown that TF's activity is dose dependent. For similar reasons, biochemical studies were virtually impossible for lack of sufficient antigen-specific raw material for purification.

In 1974, Viza and his co-workers reported that TF with known antigenic specificities could be replicated in tissue culture, using the LDV/7 lymphoblastoid cell line.[11] By incubating the cultured cells with a TF of known antigenic specificities, it was possible to retrieve, after several days of culture, a large number of cells and extract a substantial amount of TF with the same specificities as the one used for the induction.[11,20] In the late 1970s, the same group and other investigators presented evidence that specific TF obtained from mammals after immunization with a given antigen was also active in humans.[21,22]

At this time, with improving data on the impact of specific viruses on susceptible populations, the possibility of producing large amounts of standardized transfer factor for clinical trials, and the large collection of clinical information showing the absence of toxic side effects following TF administration, it appears appropriate to review the current state of the science to suggest the use of TF in the control of virus-associated malignancies.

## Viruses Involved in Cancer Pathogenesis: The Magnitude of the Problem

Viruses have been suggested in the etiology of cancer in animals for many years and they are now implicated as causative agents in more than 15% of worldwide cancer incidence.[23,24] An increasing number of infectious agents are considered to be causes of cancer in humans, one of the most recent being the Merkel Cell Tumor Virus,[7] and there are several other oncogenic agents that are under active investigation.[8–10]

Ellerman and Bang[25] first suggested that transmissible viruses can cause cancer and the avian leukemia retrovirus was followed in the next five decades by the discovery of Rous sarcoma virus,[26] mouse mammary tumor virus,[27] murine leukemia virus,[28] and others.[23] It has been estimated that 1.9 million cancer cases, or 17.8% of the global cancer burden, are attributable to infectious agents with viruses playing a predominant role.[29] The principal viral agents are the human papilloma viruses, associated with 5.2% of cancer cases, most of them cervical cancer, but with a growing list of other malignancies such as additional anogenital cancers and oropharyngeal carcinomas.

While the infectious agent with the largest cancer impact is Helicobacter pylori, associated with stomach cancer and attributed to 5.5% of cancers worldwide, this review will only focus on viruses responding to TF treatment, including oncogenic viruses such as hepatitis B Virus (HBV), one of the etiologic agents for hepatocellular carcinoma affecting 4.9% of cancer cases worldwide, and EBV, a ubiquitous virus associated with a number of malignancies including nasopharyngeal carcinoma, Burkitt's lymphoma, approximately 20% of Hodgkin's disease cases, and a small percentage of gastric carcinoma cases. However, the possible utilization of TF in the control of other virus-associated malignancies should also be considered. For example, HTLV-I, which causes adult T-cell leukemia/lymphoma is attributable to only 0.1% of cancer cases worldwide, but is a major problem in southern Japan and the Caribbean and no treatment is currently effective for this malignancy. Since the impact of virus-associated cancers is significantly greater in developing nations (27% of cases) than developed nations (8% of cases), the importance of

clinical trials possibly leading to useful treatment far less expensive than costly chemotherapeutic agents is worthy of consideration.

## The Effectiveness of Transfer Factor Against Viruses

### Studies involving DNA viruses

#### Herpes viruses

Several studies have shown that TF is effective in treating and/or preventing infections from viruses of the herpes family.

##### Herpes simplex

The effectiveness of transfer factor against herpes viruses has been documented in both animals and humans. Herpes simplex viruses HSV1 and HSV2 are ubiquitous, 75% of the adult population seem to be infected; however, only a minority of the infected individuals will suffer from it and sometimes severely so. Typically, HSV1 is responsible for labial and ophthalmic infections, HSV2 for infections in the genital area, but this distribution is not absolute. Before the advent of AIDS, genital herpes was a rapidly spreading sexually transmitted disease (STD), and probably this was one of the reasons to be one of the first human viruses treated by TF. The preliminary observations of Khan et al,[30] who reported excellent results in 17 patients with recurrent infection using a TF with not-defined antigenic specificity, were confirmed by Dwyer.[31] He compared the effect of HSV1-specific TF to a non-specific preparation for treating herpes sufferers.

At the same time, independently, Viza et al used for the first time animal virus-specific (HSV) transfer factor prepared by calf immunization for the treatment of a human viral infection, viz. HSV. The authors reported dramatic improvement in twelve patients suffering from recurrent genital herpes, resistant to several current therapies in the early 1980s, who were orally treated for a three to eight months period with bovine HSV-1 and 2-specific TF. The treatment radically reduced the intensity, duration, and frequency of the relapses. It was the first time that orally administered TF was used in a clinical trial.[32,33]

These results were confirmed in a subsequent clinical trial. Forty-four patients, 22 suffering from genital and 22 labial herpes were orally treated with HSV-1/2-specific transfer factor. TF was obtained by in vitro replication of HSV-1/2-specific bovine TF. Treatment was administered bi-weekly the first 2 weeks, and then weekly for 6 months, most patients receiving 2–3 courses. The total observation period for all patients before treatment was 26,660 days, with 544 relapses, and a relapse index of 61.2, whereas the cumulative observation period during and after treatment was 16,945 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.[34]

In another preliminary study, the activity of HSV-specific bovine TF was compared with that of Isoprinosine in the treatment of experimental herpes keratitis in 60 rabbits. The results showed a significant reduction of the formation of stromal opacities and neovessels in the TF treated animals. Furthermore, the association of TF and Isoprinosine showed a synergistic effect. In the same study, the effect of TF was compared with that of Isoprinosine in preventing relapses of ocular herpes in 17 patients. Both treatments showed a beneficial effect, TF seemingly being more efficacious in diminishing their frequency.[35] These results contrasted with those reported by Smolin et al[36] who failed to prevent or improve experimental guinea pig herpes or Candida albicans induced keratitis by using TF from blood donors, thus illustrating that antigen specificity and potency of the TF preparations is of the essence.

Additional studies in ocular disease included a clinical trial in 134 patients with recurrent ocular herpes infections, an extremely challenging problem for clinicians. This open trial compared the relapse patterns in 71 patients with keratitis, 29 with kerato-uveitis and 34 with uveitis. The patients had been followed for a total period of 189,121 days prior to TF therapy and 64,062 days after TF therapy. The total number of relapses decreased significantly from 832 prior to treatment to 89 after treatment. These clinical effects were paralleled by CMI response to viral antigens evaluated by the lymphocyte stimulation test and the leukocyte migration test. Notable were the usual absence of side effects and the more durable response of TF compared to acyclovir, which can control the infection only as long as it is administered.[37]

The clinical observations were corroborated by animal experiments to prove efficacy of TF in

protecting mice against lethal herpes simplex virus injections. Bovine HSV-1, HSV-2, and CMV TFs were prepared by immunizing calves with the corresponding virus. HSV-1 or HSV-2 specific TF was then injected at increasing amounts to 11 groups of 20 Swiss mice prior to their challenge with HSV-1 or 2. Furthermore, 14 mice received TF specific to CMV but not to HSV and were used as control of specificity. Twenty mice received saline injections instead of TF. All were subsequently exposed to lethal HSV1 or HSV2 doses. Survival of the groups treated with the HSV-specific TF was significantly higher than that of the other groups, and dose dependent. The difference was statistically significant.[38]

### Varicella-zoster

Two studies have documented the effectiveness of TF in the control of Varicella Zoster virus (VZV) infection using different approaches.[39,40] The ability of TF to prevent new VZV infection was shown by Steele et al[40] who prepared TF from the leukocytes of five adult donors convalescing from chicken pox in a controlled study of 61 children with acute leukemia with no prior history of chicken pox and a negative skin test to VZV antigen. The patients were randomized to receive either TF or a placebo and 31 of these patients could be followed for 12–30 months after exposure to VZV, 16 in the treatment group and 15 in the placebo control group. Of the 31 exposed children, 14 became clinically infected: 13 in the placebo group and one in the TF group. The affected patient in the TF group had only three skin vesicles and no systemic manifestations, whereas three patients in the placebo group had disseminated disease. There were no deaths. Skin tests to VZV antigen converted to positive in approximately half of the TF-treated patients.

Further evidence of a clinical effect was indicated by Estrada-Parra et al[39] who compared the effectiveness of TF and acyclovir in 28 patients with acute VZV infection in a double-blind study. TF was prepared from leukocytes from 1000 healthy blood donors and was given to 14 patients; another 14 were given acyclovir. Patients treated with TF had a statistically significant decrease in the duration of pain compared to the other group ($P < 0.015$). There was also an increase in CD4 cells, IFN-gamma levels and CD-4/CD-8 ratio in the TF treated group, in contrast to the acyclovir treated group who showed no laboratory changes.[37]

In a different study,[16] 12 patients who had undergone bone marrow transplantation received TF prepared from leukapheresis of five healthy adult donors convalescing from chicken pox and with very high in vitro reactivity to VZV antigen. Noting that reactivation of VZV occurs in more than 30% of patients following allogeneic bone marrow transplantation, this study was designed to see if TF could improve immunity in transplant patients. All 12 patients who had a history of primary infection with VZV remained negative to the virus by skin test and four of 12 patients subsequently developed recurrent infection, four with disseminated VZV.[16]

### Cytomegalovirus

The use of TF in active cytomegalovirus (CMV) infection has been limited, but encouraging results were reported in two case studies. Jones et al[41] reported the use of combined CMV/EBV oral bovine TF in a child with apparent combined CMV/EBV infection. This four year old boy with recurrent fever, rash, abdominal pain and arthralgia was diagnosed with the combined infection on the basis of isolation of CMV in the urine and antibody patterns consistent with acute infection coincident with the onset of illness. After treatment with oral bovine transfer factor, his clinical symptoms and viruria disappeared and specific cellular immunity to CMV, absent prior to treatment, developed.

A subsequent case report[42] of a dramatic effect in an acutely ill 7 month old child supported the likelihood that TF with anti-CMV activity can play an important role in controlling the infection. A Ghanaian child was admitted to Korle-Bu Hospital with persistent fever of more than two months duration associated with progressive loss of weight and recurrent maculopapular rashes. The child was wasted and febrile (38.5 °C) and had marked generalized lymphadenopathy and splenomegaly. Erythrocyte sedimentation rate was markedly elevated, and numerous tests to identify a specific pathogen, including multiple stool, urine and blood cultures, as well as tests for malaria and toxoplasmosis were negative. A monospot test was strongly positive and serum was frozen and sent to the United States for virological testing. On the basis of the positive monospot test, the patient was treated

with TF with known anti-EBV activity that was being used in a clinical trial in Burkitt's lymphoma (see below). After one injection of the TF derived from the lymphocytes of an EBV positive donor and replicated in the LDV/7 cell line, the patient's temperature gradually subsided to 37.5 C over a one week period with a decrease in lymphadenopathy and improvement in her appetite. One month later, after a second injection of TF, the monospot test was negative, the hepatosplenomegaly resolved and the lymphadenopathy disappeared. The patient remained disease free during the two years of follow-up. Laboratory studies in the United States, performed subsequent to the initiation of treatment, showed that the pre-TF sample had no detectable antibody to EBV, but the CMV antibody titer was 1:1028. The TF used for the treatment was subsequently analyzed for activity against CMV and HSV-2 antigens using the LMI test and was found to be highly active against these viruses.

### Oncogenic herpesviruses

Evidence of activity of TF against oncogenic herpesviruses was shown by transfer of immunity to herpesvirus saimiri (HVS), a virus harmless to its natural host, squirrel monkeys, but lethal to owl monkeys and marmosets. Pizza et al used TF to demonstrate transfer of immunity to both rhesus and owl monkeys by the use of the LMI test.[43] The initial HVS-specific TF was obtained from two adult rhesus monkeys inoculated with HVS and subsequently shown to have both humoral and cellular immunity against the membrane antigen of HVS-infected cells that had previously been shown to be important in control of HVS infection.[44] TF was prepared from the lymphocytes of the immune rhesus monkeys, replicated in the LDV/7 cell line, and injected into each of two owl monkeys and four rhesus monkeys. The two owl monkeys and three of the four rhesus monkeys developed a positive reaction in the LMI test detectable 20 months following the TF injection.[45] Demonstration of clinical effectiveness was not attempted in this study.

## Papilloma viruses

TF prepared against human papilloma virus (HPV) was reported to have significant effect in a patient with Wiskott-Aldrich syndrome, a genetically determined immunodeficiency disease.[45] In a follow-up to this report, a series of patients with this disorder were treated with TF at Stanford University[46] and in one such patient, six days following treatment with TF prepared from the leukocytes of a donor who had spontaneous regression of warts 6–12 months earlier, all wart sites developed erythema and regressed completely. Subsequently, six patients, ages 6–24, were entered into a pilot study and all three patients who had the entire series of three TF injections had regression of lesions, two of them at all body sites and the third at two of three sites in the seven week period of observation. This led to a randomized double blind study of 30 children and adolescents with varying severity of disease characterized by the size and number of warts. TF was prepared from the leukocytes of a donor whose extract had apparently resulted in regression of lesions in a Wiskott-Aldrich patient. Some dramatic regressions were observed, including complete remissions within the first weeks of the study, albeit those with improvement were evenly divided between the TF group and the placebo group. However, of the 17 with no or minimal response, 11 were in the placebo group versus six in the TF group. The authors noted the difficulty in standardizing the preparation, their inability to test the efficacy of the preparation and the possibility that immunosuppressed individuals could be more susceptible to the beneficial effect of TF. The importance of controlled double blind studies in a disease subject to spontaneous regression was emphasized.

## Hepatitis B virus

TF has been studied in therapeutic trials of HBsAg-positive chronic active hepatitis (CAH) with variable results,[47–51] each of these trials using different sources of leukocytes, including both apparently healthy donors with no history of hepatitis,[47] as well specific TF obtained from B and non-B hepatitis patients.[48–50] The largest of these three studies was a double blind investigation including nine patients with CAH in which specific TF obtained from hepatitis patients was used. A significant clinical and histological improvement was noted in the five TF-treated patients in comparison to the four placebo-treated controls. This study was followed by another trial of TF obtained from the leukocytes of four patients who

had recovered from acute Type-B viral hepatitis and replicated in vitro in the LDV/7 lymphoblastoid cell line. Twenty patients with the histological diagnosis of CAH, abnormal transaminases and stable HBs antigenemia over a twelve month period were enrolled in this study, 10 receiving TF and 10 serving as controls. Several biochemical parameters showed statistically significant improvement in the TF-treated patients compared to the controls and six of the eight patients with repeat liver biopsies showed histological improvement at the end of treatment.[50] This report emphasizes the utility of standardized TF that should be used in other studies as well as the lack of any side effects.

## RNA retroviruses

### HIV

The apparent effectiveness of TF in retroviral infections has consisted primarily of demonstrating improvement in immunologic parameters. In the first clinical study, Viza et al[52] used murine HIV-specific TF produced by mouse immunization with leukocytes from an AIDS patient and replicated in the LDV7 cell line; it was subsequently orally administered to three AIDS patients for 3–5 months. A clinical improvement and a restoration of their skin test reactivity to recall antigens together with a moderate increase of their CD4 cell counts were observed.

Carrey et al[53] used conventionally prepared "*non-specific*" TF i.e., not HIV-specific, and showed transient restoration of delayed type hypersensitivity to recall antigens of previously anergic patients and improvement of in vitro blastogenic response to phytohemagglutinin (PHA) and other ubiquitous antigens. This apparent improvement of the immune response was diminished at the end of the TF administration. Both studies showed restoration of skin reactivity.[52,53]

In a study that included 25 HIV infected patients at various stages of the disease, and all receiving antiretroviral therapy, Pizza et al used a TF prepared by mouse immunization with $2 \times 10^9$ HIV viral particles as well as with HIV-infected lymphoblastoid cells.[54] The patients were orally treated for a period between 6 and 1870 days. An increase of CD4 and CD8 was noticed respectively in 11/25 and 15/25 patients. Clinical improvement or stabilization of disease progression was noticed in 20/25 patients and a deterioration in 5/25. In 12/14 anergic patients daily TF administration restored DTH to recall antigens.

Another study compared the effect of TF to that of zidovudine. Twenty asymptomatic HIV infected patients with persistent generalized lymphadenopathy were randomly assigned to receive zidovudine alone or zidovudine together with HIV-specific TF for 6 months.[55] White blood cells CD8 lymphocytes as well as IL-2 levels increased in the TF group.

### SIV

One animal study relevant to HIV focused on simian immunodeficiency virus (SIV) that produces simian AIDS (SAIDS) in macaques, which appears to be closely analogous to AIDS in humans.[56] In this study, 19 macaques were injected with SIV and divided into 5 groups. Four groups of 4 animals were treated with TF specific to SIV obtained from the helper and/or the cytotoxic lymphocyte subpopulations as well as the total lymphocyte population of mice immunized with SIV and replicated in cell culture by the LDV/7 cells. Three animals received saline injections and were used as controls. At the end of a 108 days observation period, several biological parameters were compared in a multivariate analysis and it was found that the control group had significantly more impaired immunologic markers including the CD4/CD8 ratio and both CD4 and platelet counts; the groups treated with TF derived from or enriched with extracts from cytotoxic lymphocytes fared best.

## Studies in virus-associated cancers

### Epstein-Barr virus-associated tumors

Two of the first malignancies associated with Epstein—Barr virus, Burkitt's lymphoma (BL)[57] and nasopharyngeal carcinoma (NPC)[58] have been the target of most studies involving EBV-specific transfer factor. Brandes and Goldenberg[59] first documented transfer of immunity to EBV to NPC patients in 1974 and subsequently developed a trial of EBV-specific TF as adjuvant therapy in 100 patients with Stage III NPC treated with radical radiotherapy in collaboration with Ho.[60] The TF was prepared from young adults with a recent history of infectious mononucleosis and normal blood donors screened for high levels of anti-EBV viral capsid antibody, but with no assessment of their CMI. One half of the patients were treated with radiotherapy alone, and one half received in addition

an 18 month course of TF immunotherapy. There was no difference in disease-free survival or overall survival in the two groups.

In a different approach to study the effect of anti-EBV TF in cancer, Neequaye et al[61] used TF prepared from a donor with high EBV membrane activity replicated in the LDV/7 cell line for the prophylaxis of late relapse in a prospective study of 27 children with abdominal BL (Stage III) who had obtained complete remission. Based on extensive clinical experience in BL, Ziegler[62] had proposed two forms of relapse in this disease: a) early relapse, defined as relapse occurring within 12 weeks of completing chemotherapy, usually occurring at the same site as the original tumor, resistant to chemotherapy, and considered to be comparable to relapses in most or all other forms of cancer with emergence of resistant clones of tumor; and b) late relapse, occurring more than 12 weeks after cessation of treatment, usually seen to occur at sites different from the primary manifestations and being sensitive to chemotherapy. Late relapse was thus believed to be due to re-induction of new disease by EBV. Since BL has such a rapid rate of growth, this is a tenable hypothesis, as relapse is highly unlikely to be active and undetected for more than a few months. In this study, two patients treated with TF and two considered as controls relapsed early (12 weeks or sooner). Two of 12 TF-treated patients, and 5 of 11 controls subsequently relapsed, but the interval to first relapse was longer in the treated patients than in the controls, and no late relapse occurred while the patients were receiving TF. This study thus indicated that EBV-specific TF could play a role in preventing re-induction of EBV-induced disease by controlling virus replication.

Hodgkin's disease may also be EBV-associated, as documented by the detection of EBV RNA (EBERS) in the Reed-Sternberg cells of a high percentage of Hodgkin's lymphoma patients.[63,64] Several groups of investigators have studied the use of TF in Hodgkin's disease,[65–67] but its specificity for any particular antigen was not determined. Transfer of cellular immunity was documented by skin test conversion and increased lymphocyte response to PHA, but clinical effect was not observed.

### Human papilloma virus-associated tumors

A prospective randomized double-blind study of 60 patients with invasive cervical cancer was carried out using TF prepared from the leukocytes of the patients' husbands.[68] Although no specific attention was given to HPV as the causative agent, it is highly likely that the spouses were also HPV positive and had immunity to the virus. All patients had histologically proven Stage 1c (tumor invasion into the outer third of the wall) or higher stages and all had initially standard treatment (radical hysterectomy followed by irradiation). The patients were grouped according to histological stage, age, and the participating clinics involved in the study, and then were randomized into receiving either TF or placebo. Thirty-two patients were selected for TF treatment and 28 were designated to receive the placebo. TF and placebo were administered post-operatively and prior to radiation therapy and were continued at monthly intervals for two years unless there was a recurrence. Five (16.1%) of the TF treated patients and 11 (39%) of the placebo patients developed recurrent disease within the two year period. In analyzing the subgroups, significant differences were found in patients with Stage I disease and in patients less than 35 years of age, with minimal differences being seen in patients with Stage II disease and over 35 years of age. The in vitro lymphocyte responses to several antigens (PPD, Varidase, KLH) were significantly reduced in the post-operative period in the placebo treated patients vs. the TF group, but DTH was not found to be different between the two groups.

## Discussion

Cancer is a multi-step disorder that occurs from the combination of genetic and epigenetic abnormalities, which causes the transformation of normal cells into malignant derivatives.[69] While viruses have been shown to cause cancer in animals since the early 1900s, the link to humans did not begin until the 1960s, and the number of human oncogenic viruses continues to grow. It has been estimated that they are now implicated as causative agents in approximately 12% of cancer cases worldwide.[29]

Ellerman and Bang were the first to suggest that transmissible viruses can cause cancer in their studies leading to the identification of the avian leukosis virus.[25] At present, there are at least six human viruses believed to cause cancer, EBV, HTLV-I, HHV-6, HPV, HCV and HBV; several others are candidates.[8–10] The mechanisms of action differ significantly among

the various agents with some viruses transforming normal cells into cancer cells, such as EBV, HTLV-I and HHV-8, and others initiating cancer through intermediate pathways e.g., inflammation and fibrosis, such as HCV.

The estimated total of infection-attributable cancers in the year 2002 was 1.9 million cases, or 17.8% of the global cancer burden.[29] The principal agents are the human papilloma viruses (5.2%), the hepatitis B and C viruses (4.9%), Epstein-Barr virus (EBV) (1%), and the human immunodeficiency virus (HIV) together with the human herpes virus 8 (HHV-8) (0.9%). Other oncogenic agents, like HTLV-I, have less of an impact worldwide (0.03%), but are very important in certain regions i.e., Japan and the Caribbean for HTLV-I. There would be 26.3% fewer cancers in developing countries (1.5 million cases per year) and 7.7% in developed countries (390,000 cases) if these infectious diseases were prevented.[29]

Transfer factor is a dialysate of low MW obtained from disrupted lymphocytes capable of transferring de novo antigen specific activity to naïve lymphocytes. Numerous studies have established that TF can transfer cell-mediated immunity from a person with documented CMI to someone lacking evidence of exposure and/or immunity to the antigen. But while the in vitro evidence for its effects is considerable,[12] and the clinical evidence, if less extensive, is still impressive,[12,34,37,40] skepticism because of the lack of significant progress in unraveling its molecular structure and fully understanding its mode of action has been growing. Yet, it is worth noting that no report has ever challenged the in vitro data or that of well controlled clinical or animal studies.

Nevertheless, several enzymatic and chromatographic studies have determined numerous characteristics of the moieties with transfer activity within the lymphocyte dialysate that was initially and maybe improperly named transfer factor. The dialysate contains over 200 moieties, several carrying non-antigen specific immunological enhancing or inhibiting activities that are distinct of that of TF which is antigen-specific. Some of these molecules have been fully identified e.g., IMREG,[70,71] the presence of others is surmised from the activities of the dialysate.[72] However, only certain molecules, of MW $>$ 3500 $<$ 12000 DA, are capable of transferring antigen-specific CMI to naïve lymphocytes, and correspond to the definition of transfer factor. Furthermore, in addition to the initial antigen-specific transfer with a helper or enhancing activity by an "*inducer*" TF, evidence suggests the existence of TFs with *suppressor* function that have been named "*suppressor factor*".[73]

Semantics and definitions are of the essence in order to avoid regretful confusions with other adjuvant activities of the total dialysate such as enhancement, modulation, and non-specific inhibition of the immune response which are attributable to distinct moieties different from the TF within the dialysate. Thus the term transfer factor should be used only for lymphocyte dialysates or their purified components capable of mediating a CMI response for a known antigen that may result to an antigen-specific enhancing, inhibiting or cytotoxic effect.

TF has been purified using column chromatography and HPLC. Its biochemical characteristics have been extensively studied and they're not ordinary. Various analyses have confirmed the proteidic nature of the molecule, which is resistant to proteolytic enzymes with the exception of pronase and carboxypeptidase, but destroyed by snake venom phosphodiesterase. The prevailing model for the molecule is an oligopeptide with a ribonucleotide attached to the N terminus. As a consequence, with the N terminus blocked, the Edman degradation sequencing becomes impossible and this explains several failures in determining the AA sequence. Nonetheless, Kirckpatrick has identified a number of amino acid sequences in TF molecules,[74] but the complete sequence and the mode of action remain to be defined.

Defining antigen specificity, together with potency of the preparations used, is essential for clinical use and laboratory work. As with the use of the word *non-specific* for dialysates obtained from a pool of blood donors with untested immunological status, semantic confusions may have regretful clinical consequences and lead to erroneous conclusions.

For instance, in several occasions the data of one group of investigators were in contradiction with the findings of another team. Thus, Fog et al[75] reported failure of observing an effect in multiple sclerosis treatment by using a non-antigen specific dialysate from blood donors, whereas Basten et al in a double blind trial two years later[76] reported a beneficial effect of HSV-specific TF in slowing the progression of

disease in stage I and II patients that was confirmed by the same team 6 years later.[77] Other cases of conflicting reports of failures, probably due to the lack of testing of the extract, are related to chronic active type B hepatitis, Crohn's disease, melanoma or atopic dermatitis.

The use of colostrum as transfer factor for the treatment of a variety of pathologies is another example of confusion. Because colostrum has been proven to contain TF moieties, it has been abusively labeled *transfer factor* and commercialized as food supplement in several countries including the USA. The argument here is similar to that used against the naming and using untested human lymphocyte dialysates *non-specific transfer factor*. Indeed, the immune system of the animals making colostrum has no reason to recognize most of the human pathogens, unless it has been told by specific immunization to do so, and that is not the case. However, because of the other non-antigen specific moieties with immunological properties present therein, colostrum may have a beneficial effect on certain patients by non-specifically stimulating their immune system. The main drawbacks are that in several cases it may have no effect at all and, worst, in some cases may have undesired side effects and contrary to the expected ones.

An Egyptian study,[78] using a commercial preparation of colostrum named "*transfer factor advanced formula plus"* for the treatment of HCV infected patients illustrates this point. If improvement of certain biochemical parameters was noticed, no change in the number of NK cells or the viral load was observed. Nevertheless, the authors casually conclude that this is a *new therapeutic option* for the patients. This is comparable to previously cited contradictory reports with reference to hepatitis B treatment, one group of investigators[51] failing to reproduce the data of others.[47–50]

An additional illustration of a beneficial, but non antigen-specific activity of lymphocyte dialysates is that observed by Gottlieb's team using IMREG for restoring CMI in HIV/AIDS patients.[71]

We should therefore concur with Fudenberg that three main criteria must be respected before using TF for patients' treatment: the antigenic specificity and potency of the extract and the immune status of the recipient.[72]

If progress has suffered recently by the lack of understanding of the mode of action and subsequently by dearth of funding, past and more recent clinical results should be inspiring and encourage further clinical and structural work. For the potential of TF as a therapeutic and preventative tool is significant. Indeed, in developing countries the preventative potential of TF could play an important role. Its form is ideal for transport, storage, and administration without the typical requirements for cold preservation, and injectable administration.

For instance tuberculosis, a modern plague against which effective antibiotics are becoming scarce, could be an appropriate target for vaccination. A recent work by Fabre et al have shown that the treatment of mice infected with Mycobacterium tuberculosis with TF restored the expression of Th-1 cytokines, resulting in inhibition of bacterial proliferation and significant increase of DTH and animal survival. This beneficial effect was dose dependent. And the authors point out that murine TF, combined to conventional chemotherapy has a synergistic effect and produces significant faster elimination of lung bacteria loads than chemotherapy alone.[79]

Furthermore, alerts for new viral infections and flue epidemics appear regularly, and TF may constitute a weapon for fighting such an epidemic when it materializes.[80] The theoretical arguments discussed in this paper, have been recently considered by a Chinese team of investigators.[81]

The massive literature on TF we have briefly discussed offers valuable lessons which should be taken into consideration in determining future studies.

First, while individual case reports of success in virus-associated lesions provide useful clues, the possibility of spontaneous remissions must be kept in mind. Thus, as noted by Stevens et al, efficacy of TF in a particular virus-associated cancer requires its use in a randomized clinical trial (RCT).[46] The development of RCTs would be enhanced if the malignancy is relatively common in the area where the trial is undertaken.

Second, in regard to virus-associated malignancies, several studies suggest that TF is more likely to be effective when administered early in the course of disease or, if possible, in the pre-disease state. One example of this situation is the study by Neequaye et al[61] which appeared to show effectiveness in preventing late relapse (presumably re-induction) of EBV-associated BL with EBV-specific TF.

Third, data further suggest that long term maintenance monitored by in vitro testing may be necessary since one late relapse in the TF arm occurred four years after the cessation of TF administration.[61] Similarly, in the prospective randomized double-blind study of 60 patients with invasive cervical cancer,[68] significant differences in disease-free survival was seen in patients with Stage I disease treated with TF vs. the placebo control, but no effect was seen in patients with Stage II disease. A large prospectively randomized, double blind clinical trial of TF prepared from EBV seropositive individuals in 100 NPC patients with Stage III NPC showed no effect which, when contrasted with the apparent effectiveness in preventing late relapse from BL, further supports the need to develop clinical trials early in the oncogenic process.

Fourth, the importance of the specificity and potency of the TF should once again be pointed out. For example, in the NPC clinical trial[60] one could argue that humoral immunity doesn't always reflect the status of CMI, or the absence of blocking or suppressor factors. Determining the specificity and potency of the TF in vitro, as was performed in the study of herpesvirus saimiri focusing on virus-induced membrane antigen, is of great value and should be a prerequisite for any new study.[43]

Fifth, TF is a remarkable potential tool in terms of the absence of toxicity. Not only have there been no reports of toxic side effects, but investigators have commented specifically on the benign nature of the treatment.[46,54]

In the development of new clinical trials, it is of importance to note the advances made in preparing specific TF against the targeted oncogenic agents. Among these advances are the apparent effectiveness of orally administered TF,[32–34] the ability to replicate TF in vitro in cell lines producing standardized material for large and replicable clinical trials,[11,20] the possibility to produce TF by large animal immunization, and the consistent ability to assay for specificity and efficacy by in vitro testing.[13–17] Monitoring the efficacy of TF in producing and maintaining specific CMI against an oncogenic agent is critical, but the well documented frequently replicated technique of preparing potent and antigen-specific TF from the lymphocytes of donors with documented CMI against the target antigen[17] should pose no major problem for most laboratories.

There are four oncogenic viruses that appear to be appropriate targets for RCTs. The most apparent is HPV, the virus responsible for most virus-associated human malignancies[29] and by inference,[68] one that is amenable to control by TF. HPV is associated with a number of malignancies, including oropharyngeal and anal cancer, while cervical cancer, which should be the primary target, has an unacceptably high mortality in developing countries[82] where pap smears are either unavailable or underutilized. Treatment of early cervical cancer is extremely effective, but it would be of interest to document whether TF could cause systematic regression of pre-invasive cervical cancer in the interval between detection and treatment, which can be lengthy in some areas. Although cervical cancer is readily controlled by surgery, HPV-associated anal cancer is more difficult to detect and treat, the surgical treatment of early disease requiring anal sphincter removal and a colostomy.[83] Moreover, proof of efficacy against one HPV-associated malignancy could also be useful in controlling the other HPV-associated malignancies.

A second potential target is HTLV-I which is a major oncogenic agent in certain parts of the world.[84,85] Persistently high levels of HTLV-I antibody, a surrogate marker for high virus load, has been estimated as providing an approximately 70-fold risk of ATL compared to those with the lowest titers.[86] Quantitative proviral DNA and antibody levels have been monitored in natural history studies of HTLV-I infection,[87] and persistently high levels of HTLV-I, a surrogate marker for high virus load, has been estimated as providing an approximately 70-fold risk of adult T-cell leukemia/lymphoma (ATLL), compared to those with the lowest titers.[85] Therefore, clinical trials of HTLV-I specific TF in a study of subjects with high titers in the Caribbean or Japan, where the virus is endemic,[84,85] should document whether control of the virus is possible and subsequently whether ATLL can be prevented.

The prevention of malignancy with early TF administration is relevant in the management of the other two viruses, HBV and HCV, and their associated cancer, hepatocellular carcinoma (HCC). Beasley[3] noted the association of HBV infection with HCC and developed a prospective study in Taiwan using HBV vaccine that was first deployed as an important tool to control chronic HBV infection, a risk factor for

chronic hepatitis and cirrhosis, known to predispose to HCC. Recent data indicate the effectiveness of this vaccine in reducing the incidence of HCC in Taiwanese,[88] an extremely important demonstration of the possibility of HBV-associated HCC control, but the observation that the vaccine may not be effective in offspring of highly infectious mothers suggests a possible role for HBV-specific TF in those cases. HCV is a particularly difficult problem since there is no vaccine against this virus and antiviral therapy is often ineffective, has significant side effects, and requires long term treatment.[89] Infections with HCV peaked in the United States in the early 1970s and 1980s but this virus remains a significant problem in many countries.[90] Because of the long latent period between HCV infection and liver cancer, a rise in the incidence of liver cancer can be expected and indeed has been suggested in recent data in the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute.[91]

In summary, there is considerable promise in investigating virus-specific TF as a tool in reducing the impact of virus-associated cancers. It has a different role to play compared to chemotherapy, which is definitely more expensive, often palliative, certainly not preventive, and clearly more toxic.

## Abbreviations

ATLL, Adult T-Cell Lymphoma Leukemia; BL, Burkitt's lymphoma; CAH, Chronic active hepatitis; CMI, Cell-Mediated Immunity; CMV, Cytomegalovirus; DTH, Delayed Type Hypersensitivity; EBV, Epstein Barr Virus; HBV, Hepatitis B Virus; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C Virus; HHV-6, Human Herpes Virus-6; HPV, Human papilloma virus; HSV, Herpes simplex virus; HTLV-1, Human T-cell Lymphotropic Virus-1; IFN, Interferon; IL2, Interleukin 2; LMI, Leukocyte Migration Inhibition Test; LST, Lymphocyte stimulation test; MMTV, Mouse Mammary tumor virus; NPC, Nasopharyngeal Carcinoma; RCT, randomized controlled trials; TF, Transfer Factor; VZV, Varicella Zoster Virus; WBC, white blood cells.

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