Review Article

Transfer Factor: an Overlooked Potential for the Prevention and Treatment of Infectious Diseases

(AIDS / cancer / candidiasis / cell-mediated immunity / flu / fungal / herpes / HIV / immunodeficiencies / infections / leishmaniasis / mycobacterial / parasitic / transfer factor / tuberculosis / vaccines / varicella / virus)

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Abstract. Transfer factor (TF) is a low-molecular-weight lymphocyte extract capable of transferring antigen-specific cell-mediated immunity (CMI) to T lymphocytes. It has been used successfully as an adjuvant or primary therapy for viral, parasitic, fungal, and some bacterial infections, as well as immunodeficiencies, neoplasias, allergies and autoimmune diseases. From the list of infections that seem to respond noticeably to transfer factor, those due to viruses of the herpes family are particularly remarkable. Indeed, for these viruses it was shown that TF can prevent infection or relapse, acting as a CMI vaccine. Data also suggest its possible use for adjuvant treatment and probably prevention of two currently widespread infections: tuberculosis and AIDS. Furthermore, TF has an interesting potential: answering the challenge from unknown pathogenic agents, a black box effect permitting production of antigen-specific TF to a new pathogen, even before its identification. It thus seems that the preventative potential of transfer factor is as important as its therapeutic one, both discussed in this review.

Received October 22, 2012. Accepted October 29, 2012.

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Abbreviations: BB – *Borrelia burgdorferi*, BL – Burkitt's lymphoma, CBO – Congressional Budget Office, CMI – cell-mediated immunity, CMV – cytomegalovirus, CTL – cytotoxic T lymphocytes, DLE – dialysable lymphocyte extract, DTH – delayed type hypersensitivity, EBV – Epstein-Barr virus, HHV – human herpes virus, HIV – human immunodeficiency virus, HSV – herpes simplex virus, LMI – leukocyte migration inhibition, MW – molecular weight, NPC – nasopharyngeal carcinoma, PHA – phytohaemagglutinin, SAIDS – simian AIDS, SIV – simian immunodeficiency virus, STD – sexually transmitted diseases, TF – transfer factor, TB – tuberculosis, VZV – varicella zoster virus.

Introduction

The threat of infectious diseases has not vanished; on the contrary, in the last half century new pathogens have been emerging or discovered. Since the identification of *Legionella pneumophila* as the cause of Legionnaires' disease in 1976, the list of newly identified pathogens lengthens to now include the Ebola and SARS viruses, as well as HIV, SIV, FLV, HTLV I and II, the new herpes viruses (HHV-6, 7, & 8) and the multiple recombinations/mutations of the flu virus. To the natural emergence of pathogens, one has to include terrorist-made bio-threats that are now a plausible probability. Indeed, if for instance the smallpox virus has been declared eradicated, it remains in hibernation in laboratory freezers, and its accidental or criminal release has still the potential of producing worldwide devastation. Furthermore, because microorganisms are continuously becoming more resistant to antibiotics and antivirals, the need for new defensive agents is urgent.

The situation in developing countries is worse than that in the developed world. For instance, HIV infection and tuberculosis (TB), often combined in a lethal association, are rampant, and the toll is millions of deaths per annum. In the present review, we will explore the possibilities of using transfer factor (TF) for the prevention and/or treatment of infections, after reviewing past work and considering new approaches.

Transfer factor is an extract obtained from immune lymphocytes capable of transferring antigen-specific information for cell-mediated immunity (CMI) from an immunized donor to a naïve recipient. The first observations postulating its existence and establishing its concept date back to the early 1950s (Lawrence, 1954,

1955). Lawrence showed that delayed type hypersensitivity (DTH) to a given antigen could be transferred from one individual to another via acellular extracts obtained from the leucocytes of immunized donors. He assumed that this adoptive transfer of immunity was due to the molecules that he named transfer factor, and he surmised that their molecular weight (MW) was less than 12,000 Da, as they filtered through a standard dialysis bag. Since that time, TF has been prepared by disrupting lymphocytes and dialysing the lysate. The dialysed material thus obtained was used for *in vitro* tests and for *in vivo* clinical or animal studies (Jeter et al., 1954; Lawrence, 1955).

Further studies indicated that the activity is carried by molecules of ca 5000 Da (> 3500 < 5000), and biochemical analysis suggested that the biological activity was carried by an oligoribonucleopeptide with the ribonucleotide attached to the amino terminus of the peptide. As a result, conventional methods of sequencing the peptide using the Edman reaction have so far been without success. Additional progress was made with a partial amino acid sequence identification (Kirkpatrick, 2000), but this report remains unconfirmed, and no further reports have been published in this area. Besides the lack of precise identification of the structure, the mechanism of action at the molecular level remains so far hypothetical. In order to resolve these uncertainties, a fresh approach is called for.

However, and notwithstanding the biochemical uncertainties, since Lawrence's (1954, 1955) original observations, over fifteen hundred publications have established that dialysable extracts from lymphocytes of immune donors can transfer antigen-specific CMI information *in vitro* to naïve lymphocytes and *in vivo* to patients and experimental animals (for instance: http://www.aosp.bo.it/files/tf_extensive_bibliography_edit_2_1.pdf).

Because CMI plays a crucial role in the control of infections, as well as cancer, TF has been used over the past 35 years, sometimes with dramatic success, for the treatment of viral parasitic, fungal, mycobacterial infections, and some cancers, as well as for the prevention of certain viral infections. Nonetheless, in spite of extremely promising clinical results, research in this field has been impeded by theoretical considerations as well as practical problems. For instance, for many years TF was prepared from pools of blood donors' lymphocytes, and seldom from donors with high probability of carrying the desired antigenic specificity, e.g. from patients' household contacts. Results obtained by selecting immune donors were impressive, sometimes spectacular, whereas results achieved with extracts from random blood donors were inconsistent: dialysate produced from random donors usually lacked the required antigenic specificity and/or potency. However, new sources of antigen-specific TF were developed in the '70s. Viza et al. (1975, 1982) reported that TF with known antigenic specificities could be "replicated" in tissue culture by a lymphoblastoid cell line, and in the late '70s, evidence was presented that antigen-specific TF obtained from mammals after immunization with a given antigen was active in humans (Boucheix et al., 1977; Vich et al., 1978).

The rationale for the *in vitro* apparent replication stems from the original observations by Lawrence (1954, 1955). He had shown that TF carrying a donor's antigenic specificity can be retrieved from the lymphocytes of a naïve recipient injected with the donor's TF. The lymphocytes of the recipient are apparently acting as an efficient copier, integrating the specificity of the injected TF, and the TF recipient is thus becoming an effective donor. The assumption of Burnet (in Fudenberg and Fudenberg, 1989) that TF replicates when injected into an organism seems to be correct by means of recruitment of naïve cells. However, if an antigen-specific TF is able to be used for the information of naïve lymphocytes, it follows that the immune information within the lymphocytes that have already recognized epitopes of a new pathogen can be transferred by the TF, before scientists or physicians have identified that pathogen. This is what Viza has called the "*black box effect*" (in Pizza et al., 2006). It can be used for production of antigen-specific TF, even if the identity of the antigen is unknown, for instance by injecting laboratory animals with TF from patients' household contacts, or by inducing an adequate cell line with such TF. This approach should contribute to the solution of the availability problems, including non-identified pathogens (Pizza et al., 2006). Moreover, animal immunization with unidentified or partially identified microorganisms may also be utilized for the same purpose: production of a TF for new antigen specificities. It was by this method that the first HIV-specific TF was produced: mice were immunized with lymphocytes from an AIDS patient, when the existence of the HIV virus was only surmised (Viza et al., 1987).

Clinical and animal studies: a brief review

I. Viral infections

1. Viral hepatitis

Clinical trials using TF to treat HBsAg chronic active hepatitis produced variable results (Tong et al., 1976; Jain et al., 1977; Shulman et al., 1979), as the source of TF was not standardized, and sometimes it was HBsAg-non-specific (Tong et al., 1976). However, when HBsAg-specific TF obtained from the leucocytes of patients who had recovered from acute type-B viral hepatitis and replicated *in vitro* in the LDV/7 lymphoblastoid cell line was used, the results confirmed the effectiveness of TF, showing a net improvement of the treated patients versus the controls as assessed by several biochemical parameters and by liver biopsies (Pizza et al., 1979; Roda et al., 1985).

2. Herpes viruses

a) Herpes simplex virus (HSV)

The efficiency of TF to prevent or control herpes simplex virus (HSV) infections has been shown by several clinical trials and animal studies. In the late '70s, sexually transmitted diseases (STD), viz. genital herpes, were rapidly spreading. However, if approximately 75 % of the adult population carries HSV-1 and/or 2, only a minority of the infected individuals will suffer from the manifestations of the infection, the immune system being able to control the expression of the virus. Hence the rationale for using TF.

First, Khan et al. (1981) used a TF of unknown antigenic specificity for the treatment of 17 patients with recurrent herpes bouts and observed encouraging results. These observations were confirmed by Dwyer (1983), who in addition compared the effect of HSV-1-specific TF to a non-antigen specific preparation. At the same time, Viza et al. (1983) used animal virus-specific TF (prepared by calf immunization with live HSV-1&2) for the treatment of a human viral infection. They reported dramatic improvement in 12 patients suffering from recurrent genital herpes resistant to antiviral therapies available in the early '80s (Viza et al., 1983; Rosenfeld et al., 1984). Subsequent clinical studies confirmed these observations. In an open study, Pizza et al. (1996b) treated 22 patients suffering from genital herpes and 22 from labial herpes. HSV-1/2-specific TF obtained by *in vitro* replication of the bovine TF used in the previous study was administered orally. 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 relapse index of 21.4, a highly significant result.

In a different open trial, 134 patients with recurrent ocular herpes infections (keratitis, kerato-uveitis, or uveitis) were treated with the same tissue culture-replicated bovine TF. The total number of relapses decreased significantly after the treatment. The clinical results reflected the CMI response to viral antigens (Meduri et al., 1996). The usual absence of side effects is worth noting, since the patients in both studies had received treatment and were monitored for extremely long periods (Meduri et al., 1996; Pizza et al., 1996b). The long-lasting effect of TF is also worth mentioning, compared to that of antivirals that can only control the infection as long as they are administered.

These data obtained in open clinical trials were supported by animal studies. Bovine HSV-1, HSV-2, and CMV-specific TFs were prepared by calf immunization with the corresponding viruses. The extracts were used to protect mice against lethal challenge with HSV. Twenty mice received saline injections instead of TF, 14 received TF specific to CMV, but not to HSV, and were used as control of specificity. All animals were subsequently exposed to lethal amounts of HSV-1 or HSV-2. Survival of the groups treated with the HSV-specific TF was significantly higher than that of the other groups, and dose dependent (Viza et al., 1986).

b) Varicella zoster virus

The most important clinical studies showing the ability of a virus-specific TF to prevent a viral infection are that of Steele's group. TF from the leucocytes of five adult donors convalescing from chickenpox was used in a controlled study that included 61 children with acute leukaemia, with no prior history of chickenpox and a negative skin test to varicella zoster virus (VZV) antigen. The patients were randomized, receiving either TF or a placebo. Sixteen patients in the TF group and 15 in the placebo were exposed to VZV, and most of them had a rise in antibody titre. However, only one patient in the TF group became infected vs. 13/15 in the placebo group, a highly statistically significant difference (Steele et al., 1980). Furthermore, in the patients treated with TF and exposed to VZV without acquiring chickenpox, the titre of antibody to the virus was equal to that in the patients receiving placebo who became infected with chickenpox, thus confirming the assumption that TF-conferred CMI is responsible for the protection. Moreover, TF converted negative skin tests for VZV to positive in approximately half the recipients. For the first time, it was clearly shown that adoptive immunization by TF protects non-immune persons against a viral infection.

In a second study, 12 patients who had undergone bone marrow transplantation received TF prepared from five healthy adult donors convalescing from chickenpox with high *in vitro* reactivity to VZV antigen. This study was intended to confirm that TF can improve immunity in such patients, as VZV reactivation occurs in more than 30 % of patients following allogeneic bone marrow transplantation. All 12 patients who had a history of primary infection with VZV remained negative to the virus by skin testing. However, four subsequently developed recurrent infection, two with disseminated VZV. The immunosuppressive treatment used in bone marrow transplantation may be responsible for this result (Bowden et al., 1985).

Eighteen years later, in a double-blind study, Estrada-Parra et al. (1998) compared the efficacy of TF and acyclovir in 28 patients with acute VZV infection. TF was prepared from leucocytes from 1000 healthy blood donors and was given to 14 patients, whereas another 14 were treated with acyclovir. The TF patients had a statistically significant decrease in the duration of pain compared to the acyclovir group. Furthermore, there was an increase of CD4 lymphocytes, interferon γ levels and CD4/CD8 ratio in the TF-treated group, in contrast to the acyclovir patients who showed no laboratory changes.

c) Cytomegalovirus

A case report by Jones et al. (1981) described the effect of combined EBV/CMV-specific bovine TF orally administered to a child with apparent combined EBV/

CMV infection. The treatment produced a dramatic clinical improvement with disappearance of viruria and development of antigen-specific CMI to CMV that was absent prior to treatment. Another case report was published by Nkrumah et al. (1985) and relates the dramatic effect of TF in an acutely ill 7-month-old child, wasted and febrile with marked generalized lymphadenopathy and splenomegaly. After one injection of an EBV/CMV--specific TF, derived from the lymphocytes of an EBV/CMV-positive donor and replicated in the LDV/7 cell line, the patient's temperature gradually subsided and the lymphadenopathy decreased. After a second TF injection, the monospot test became negative, the hepatosplenomegaly resolved and the lymphadenopathy disappeared. The patient remained disease-free during the two years of follow-up.

d) Epstein-Barr virus

Epstein-Barr virus (EBV) is a major cofactor, if not the causative agent, of two malignancies: Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). Thus, most studies using EBV-specific TF have targeted these tumours. For instance, Brandes and Goldenberg (1974) designed a trial using EBV-specific TF as an adjuvant treatment in 100 patients with Stage III NPC. TF was prepared from young adults with a recent history of infectious mononucleosis and 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, the other half received an 18-month course of TF immunotherapy. No difference in disease-free survival or overall survival in the two groups was observed (Goldenberg et al., 1985).

In a prospective study, Neequaye et al. (1990) used TF prepared from a donor with high EBV membrane activity and replicated in the LDV/7 cell line for the treatment of 27 children with abdominal BL (Stage III) in complete remission to protect from late relapses, as late relapses are believed to be caused by re-induction of new disease by the virus (Ziegler et al., 1972). Two TF-treated patients and two controls relapsed early, whereas 2/12 TF-treated patients and 5/11 controls relapsed later, but the interval to the first relapse was longer in the TF-treated patients than in the controls. No late relapse occurred under TF immunotherapy. These data suggest that EBV-specific TF may play a role in preventing re-induction of EBV-induced disease.

e) Hodgkin's lymphoma

Because EBV is probably an actor in Hodgkin's disease (Jarrett et al., 1996; Gulley et al., 2002), some investigators have studied the use of TF in such patients (Khan et al., 1975; Ng et al., 1975; Hancock et al., 1988). However, the TF used was antigen-non-specific. Skin test reactivity was markedly enhanced in some patients receiving the TF, as opposed to placebo, but other immunological assessments showed no significant differences between the groups and no clinical effect was observed (Khan et al., 1975).

3. Retroviruses

a) HIV and AIDS

In the first clinical study, murine HIV-specific TF was produced by mouse immunization with leucocytes from an AIDS patient and replicated in the LDV/7 cell line. It was orally administered to three AIDS patients for 3–5 months. A clinical improvement, a restoration of their skin test reactivity, and a moderate increase of their CD4 cell counts were observed (Viza et al., 1987). Carrey et al. (1987) observed a transient restoration of DTH to recall antigens of previously anergic patients by the injection of conventionally prepared, non-HIV-specific TF. The *in vitro* blastogenic response to phytohaemagglutinin (PHA) and other ubiquitous antigens was also increased. This apparent improvement of the immune response was diminished at the end of the TF administration. Similar observations were made using IMREG (Gottlieb, 1991; Gottlieb et al., 1991), low-MW moieties present in the lymphocyte dialysates that exert a non-antigen-specific effect on CMI and increase DTH, for the treatment of AIDS patients. These authors reported restoration of DTH to recall antigens and expression of IL-2 receptor on T lymphocytes.

In a subsequent study that included 25 HIV-infected patients at various stages of the disease, all receiving antiretroviral therapy, Pizza et al. (1996a) used TF prepared by mouse immunization with HIV viral particles as well as HIV-infected lymphoblastoid cells and reproduced *in vitro*. The patients were treated orally for a period between 60 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 deterioration in 5/25 patients. In 12/14 anergic patients, daily TF administration restored DTH to recall antigens. An additional study using the same TF compared its effect to that of zidovudine. Twenty asymptomatic HIV-infected patients with persistent generalized lymphadenopathy were randomly assigned to receive zidovudine or zidovudine together with HIV-specific TF for six months. White blood cells, CD8 lymphocytes as well as IL-2 levels increased in the TF group, suggesting activation of the Th1 cytokine pattern (Raise et al., 1996).

b) Simian immunodeficiency virus and simian AIDS

Animal studies for investigating the HIV infection are difficult and expensive to implement. The simian immunodeficiency virus (SIV) infection, producing simian AIDS (SAIDS) in macaques, is the most accessible model, and presents similarities to that of HIV. Thus, SIV-specific TF was used to investigate its effect on 19 macaques injected with SIV. After the SIV injection, the animals were divided into five groups. Four groups of four 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 reproduced in cell culture by the LDV/7 cells. Three animals received sa-

line injections and were used as controls. In a multivariate analysis, at the end of a 108-day observation period, it was found that several biological parameters of the control group were significantly different from those of the others. In a univariate analysis, the CD4/CD8 ratio, as well as the CD4 cells and platelet counts, showed significant variations between the treated and the control groups. It is worth noting that the groups treated with TF derived from or enriched with extracts from CD8 T cytotoxic lymphocytes fared best (Viza et al., 1988).

4. Human papilloma viruses

In a prospective randomized double-blind study, 60 patients with invasive cervical cancer received TF prepared from the leucocytes of the patients' husbands (Wagner et al., 1987). All patients had histologically proven Stage 1c tumour invasion and all had initially standard treatment (radical hysterectomy followed by irradiation). The patients were randomized into two groups: 32 patients receiving TF and 28 receiving placebo. Both TF and placebo were administered post-operatively, but prior to radiation therapy. The treatment was continued at monthly intervals for two years, except when there was a recurrence. Five (16.1 %) patients in the TF group and 11 (39 %) of the placebo group developed recurrent disease within the two-year period. The *in vitro* lymphocyte responses to recall antigens were significantly reduced in the post-operative period in the placebo-treated patients, contrary to the TF group.

II. Parasitic infections

1) Leishmaniasis

Cutaneous leishmaniasis is a parasitic infection usually self-limiting in humans that in some patients may persist for several years. TF seems to be able to control the disease, as Sharma et al. (1979) and Delgado et al. (1981) have shown. In Sharma's initial study, intensive treatment with specific TF for several months produced dramatic healing of the lesions in three patients whose disease had persisted for 8–30 years. Similar results were obtained in a subsequent study by Delgado et al. (1981). They administered TF prepared from leishmania-responsive healthy donors to seven patients without antigen-specific CMI response. Four of the seven patients with acute leishmaniasis had a complete resolution of their disease, accompanied by conversion to antigen responsiveness. The other three patients, suffering from the disease for at least 10 years, didn't respond.

2) Cryptosporidiosis

Fourteen patients with AIDS and symptomatic cryptosporidiosis were treated with an antigen-specific TF prepared from lymph node lymphocytes of calves immunized with cryptosporidia or a nonspecific TF prepared from non-immunized calves. Six of seven patients given specific TF gained weight and had a decrease in bowel movement frequency, with eradication of oocysts from stool in five patients, whereas six of seven patients given nonspecific TF showed no decrease in bowel movement, four no clearing of oocytes from the stool, and five continued to lose weight. Five of them were subsequently treated with specific TF, and four had a decrease in bowel movement frequency and a significant weight gain, with eradication of oocytes from the stool in two of them (McMeeking et al., 1990).

3) Echinococcosis

A positive effect of TF on the immune response of mice infected with *Echinococcus multilocularis* and treated with albendazole was observed. TF administration increased the parasite-suppressing proliferative response on T and B lymphocytes of infected mice. The production of IFN-γ (Th1 cytokine) after TF or TF and albendazole therapy was significantly higher 6 to 12 weeks post infection, and during that time significantly inhibited IL-5 synthesis (Th2 cytokine) (Dvoroznáková et al., 2009).

III. Fungal infections

1) Chronic mucocutaneous candidiasis

This is an immunodeficiency syndrome characterized by chronically relapsing *Candida albicans* infections. Several publications have reported that it responds to TF treatment (Fudenberg et al., 1974; Kirkpatrick and Greenberg, 1979; Masi et al., 1996). For instance, Masi et al. (1996) reported significant clinical improvement in all but one of the fifteen patients treated, and it was also shown that Candida-specific TF increases the patients' immune reactivity to Candida antigens.

2) Coccidioidomycosis

It is a fungal disease caused by *Coccidioides immitis* or *C. posadasii*, endemic in certain parts of USA and Mexico, and it seems to respond to TF therapy. Graybill et al. (1973) treated three patients with progressive coccidioidomycosis, inducing in two of them prolonged clinical remission. Moreover, conversion of the skin tests and the lymphocyte transformation response to coccidioidin was observed. Similar results were obtained by Catanzaro et al. in 1974.

IV. Mycobacterial infections

1) Tuberculosis

Probably one of the most rapidly expanding infections today is that of *Mycobacterium tuberculosis*, whose emerging resistant strains make treatment continuously more difficult. TF was used as early as 1973 for the treatment of a patient with progressive primary tuberculosis who failed to respond to conventional therapy, and in whom a defect in CMI to *M. tuberculosis* was shown by *in vivo* and *in vitro* studies. The administration of TF resulted in the development of CMI reactivity, accompanied by impressive improvement of the clinical condition, thus suggesting that TF in conjunction with conventional therapy was instrumental in the resolution of the patient's infection (Whitcomb and

Rocklin, 1973). Another case report described successful treatment with TF of a patient with progressive TB, refractory to chemotherapy with eight drugs. Clinical recovery was accompanied by rapid conversion of cell-mediated immunodeficiency, confirming the role of TF as an adjuvant to chemotherapy of TB in immunodeficient patients (Rubinstein et al., 1977).

More recently, a Mexican team of investigators have shown that treatment of mice infected with *Mycobacterium tuberculosis* with a murine tuberculosis-specific TF restored expression of the Th1 cytokine pattern and resulted in inhibition of bacterial proliferation, significant increase of DTH, and animal survival (Fabre et al., 2004). The specific TF was produced from tuberculous BALB/c mice following intra-tracheal infection. As for the HSV-specific TF mouse model (Viza et al., 1986), the effect of TF was dose-dependent. Furthermore, when TF was combined with conventional chemotherapy, it showed a synergistic effect, producing significantly faster elimination of lung bacteria loads than chemotherapy alone. Considering the present re-emergence of this disease, with appearance of antibiotic-resistant strains, TF may become, in the years to come, a significant actor, not only as an adjuvant to chemotherapy, but also playing a role in the prevention of the infection.

2) Lepromatous leprosy

Manifestations of the disease caused by *Mycobacterium leprae* is characterized by the absence of CMI responses to the lepromin antigen and to the mycobacterium itself (Myrvang et al., 1973). Additional CMI defects include reduction in peripheral and lymph node T cells, impaired response to mitogens, and anergy to common recall antigens. These defects are usually reversed by anti-mycobacterial therapy, although apparently cured patients often remain anergic to lepromin. One study of the effect of lepromin-specific TF described induction of lepromin responsiveness in 6/9 patients following one TF injection (Bullock et al., 1972). In an another study, four patients treated with a 12-week course of antigen-specific TF, in conjunction with anti-mycobacterial therapy, showed enhanced elimination of mycobacteria from lesions, and their skin tests became lepromin-positive (Hastings et al., 1976). However, the clinical improvements observed are not convincing, and larger clinical trials with antigen-specific TF are necessary to confirm that patients may benefit from such therapy (Katoch, 1996).

3) Other mycobacterial infections

Two reports describe the effect of TF on *Mycobacterium fortuitum pneumonia* refractory to antibiotic therapy (Wilson et al., 1982) and on drug-resistant *Mycobacterium xenopi* infection (Dwyer et al., 1983). Antigen-specific TF, prepared from leucocytes of three donors with high skin-test reactivity to *Mycobacterium xenopi* antigen, was administered to a patient suffering from destructive pulmonary infection with this microorganism resistant to antibiotics. The antigen-specific TF, in contrast to antigen-nonspecific TF, caused rapid and prolonged improvement of both the pulmonary disease and the immunological status of the patient, as well as restoration of the skin-test reactivity to *M. xenopi* antigen. Subsequently, and because of cross-reactivity between *M. xenopi* and *M. tuberculosis*, TF obtained from tuberculin-positive donors was also successfully used (Dwyer et al., 1983).

Discussion

It is well known that cell-mediated immunity plays a crucial role in controlling viral and mycobacterial infections. Both types of pathogens are direct or indirect health threats, capable of creating multifaceted associated pathologies, as well as future potential perils due to the emergence of new strains or the chemoresistance of the existing ones.

For instance, it is worth remembering that nearly 1.9 million cancer cases (17.8 % of the total cancer cases) are caused by infectious agents, primarily viruses, where inflammation plays a crucial role. Thus, HPV is responsible for over 5 % of cancers, e.g. cervical, anogenital, and oropharyngeal (Parkin, 2006), while the hepatitis viruses generate cancers of the liver. HIV is another virus whose spread has been difficult to curtail, and its association with tuberculosis makes TB deadlier than in HIV-free patients. Furthermore, the numbers of TB cases and *M. tuberculosis* resistant strains are also ever-increasing.

Several studies, briefly mentioned in this review, suggest that TF is capable of enhancing the existing CMI responses and/or of transferring information to recognize and induce a novel response to various pathogens. It should thus be considered as an instrument with significant potential, not only for treating, but also for preventing pathologies caused by them, viz. cancer (Levine et al., 2011). Furthermore, its pleiotropic effects shouldn't be overlooked, as the lymphocyte extract contains moieties with both enhancer and suppressor activities, carrying several antigenic specificities (Lawrence, 1995), as well as nonspecific immunoactive molecules such as IMREG. For example, TF may act in patients with virally induced cancers by enhancing their ability to eliminate the corresponding virus and/or by increasing their capacity to recognize and destroy the newly formed cancer cells. At the same time, by enhancing suppressor cell activities, it should contribute to controlling the ensuing inflammatory processes.

However, in spite of numerous publications showing dramatic clinical improvements, impressive animal and *in vitro* data (http://www.aosp.bo.it/files/tf_extensive_bibliography_edit_2_1.pdf), several drawbacks have beset the wide use of TF. And paradoxically, one of the main conceptual problems is probably its success in treating several types of unrelated pathological conditions. Indeed, biochemically ill-defined moieties with a panacea-like effect arouse defiance today, especially if their mode of action remains hypothetical, if not contro-

versial, and their toxicity is nil. The placebo effect haunts medical investigators, and the implicit consensus is to ignore interesting data than to get involved with a fluke. However, its panacea-like activity can simply be accounted for by the very nature of the immune system, evolved to recognize and resist all sorts of exogenous or endogenous invaders, even utterly unknown and unpredictable, bearing alien epitopes. The recruitment of naïve T lymphocytes to face new pathogens is one of the immune system's essential and miraculous functions, as is its ability to produce antibodies recognizing almost every possible antigen.

I. Specificity and potency

1) Causes for inactivity

Failure to reproduce clinical results in some cases has been used as an argument against TF. However, when data permit, a closer examination of the published reports shows that no well-designed and controlled clinical studies have ever failed to be reproduced. However, to ensure consistent results, several parameters must be taken into account.

The first and probably the most important is the antigen specificity of the extract. Calling dialysable lymphocyte extracts (DLE) "transfer factor" has created enormous confusion. Indeed, the extracts are often obtained from a pool of donors and reflect collective CMI memory that may or may not contain the desired antigenic specificity. They contain hundreds of molecules, most with nonspecific immunological activity, as well as strictly speaking transfer factor moieties, with inducing and suppressing activities that cover a large spectrum of antigenic specificities, but not necessarily the ones appropriate for treating the particular patient and not necessarily in sufficient quantities. TF, on the other hand, not only enhances pre-existing immunological memory, but it also transfers new antigen-specific information and generates *de novo* memory for antigens that the recipient's immune system has never encountered or was incapable of recognizing and/or mounting an effective response.

Thus, the absence of effect observed in some clinical trials and the reason why in some instances clinical data of one group of investigators contradict that of another may be explained by the use of untested and/or inactive preparations. For instance, Fog et al. (1978), using a non-antigen-specific DLE from blood donors, reported that TF had no effect in multiple sclerosis patients, an observation contrasting with that of Basten et al. (1980). In a double-blind clinical trial, the latter reported a beneficial effect with HSV-specific TF, capable of slowing the progression of disease in stage I and II patients. These data were confirmed six years later (Frith et al., 1986). Similarly, one group of investigators (Tong et al., 1976) produced data contradicting that of others (Jain et al., 1977; Pizza et al., 1979; Shulman et al., 1979; Roda et al., 1985) in treating chronic active hepatitis B. Other cases of conflicting reports of failure, probably due to the lack of testing for specificity and potency of the DLE, relate to Crohn's disease, melanoma, and atopic dermatitis.

Potency of the extract is the second important criterion for an active TF, and depends on the condition of the donors, who should present a sufficient CMI and lack suppressor factors. Furthermore, the end result will also depend, as with most medications, on the immune status of the recipient. For instance, it would be a forlorn attempt trying to restore CMI in patients receiving an immunosuppressive treatment. Therefore, defining antigen specificity and potency of TF is of paramount importance for planning clinical trials and animal studies.

Up to now, there are only two ways to obtain TF with the desired specificity and potency: animal immunization and *in vitro* replication of a TF with known specificity by an adequate lymphoid cell line and probably by fresh lymphocytes in culture. Lymphocyte extracts obtained from donors who may be lacking the desired antigenic specificity shouldn't be named "transfer factor", nor *a fortiori* colostrum obtained from naïve animals, and should not be used for therapy.

It should be mentioned here that non-antigen-specific IMREG-like moieties play certainly a role in synergistically enhancing the inducer or suppressor activities of the DLE. It is even possible that some of the observed pleiotropic effects of the DLE are synergistically augmented or inhibited, and could even be lost if pure TF molecules were to be used. Thus, the identification and the role of all immunologically active molecules present in the DLE are of importance and should be undertaken, once the structure of the strictly speaking TF moieties has been defined.

2) Transfer factor versus colostrum

If extracts obtained from a vast pool of blood donors with untested immunological status offer a low probability of containing a desired antigenic specificity in sufficient amounts, colostrum obtained from non-immune animals offers none. And if naming randomly obtained non-antigen-specific lymphocyte dialysates "transfer factor" is an incorrect extrapolation, creating semantic confusions that can lead to erroneous conclusions and ineffective treatments, calling colostrum "transfer factor" and use it for the treatment of a variety of pathologies is abusive.

Indeed, colostrum has been shown to contain TF-like moieties, but it has been abusively labelled "transfer factor", and commercialized as food supplement in several countries, including the USA. Obviously, the immune system of animals making colostrum has no reason to recognize most of the human pathogens, unless it has been instructed by specific immunization to do so. This is not the case, as regulatory agencies in most countries forbid such procedures. Thus, using for instance colostrum from HIV-naïve cows to combat the HIV infection cannot be expected to be efficacious, as the cow has no reason to produce an HIV-specific TF to protect its calf, the virus being innocuous in that species.

Nonetheless, because other non-antigen-specific moieties with enhancing immunological activity are present therein, colostrum may possibly exert a beneficial effect on certain patients by non-specifically stimulating their immune system. For instance, IMREG (Sinha et al., 1988) was used for restoring CMI in HIV/AIDS patients (Gottlieb et al., 1991), and probably IMREG is present in colostrum.

Recently, an Egyptian team of investigators used a commercial preparation of colostrum named "Transfer Factor advanced formula plus" for the treatment of HCV-infected patients (El-Moety et al., 2008). The results were at best inconclusive. Improvement of certain biochemical parameters was noticed, but no change in the number of NK cells or the viral load had occurred. Nevertheless, the authors concluded that this is a "new therapeutic option" for the patients, ignoring previous contradictory reports with reference to hepatitis B treatment (Tong et al., 1976; Jain et al., 1977; Pizza et al., 1979; Shulman et al., 1979; Roda et al., 1985). Such hasty conclusions and unwarranted statements from studies with uncertain results fuel the TF controversy without offering new insights. Indeed, when the existence of TF is doubted by some scientists because its biochemical structure remains undefined, it is regrettable to add confusion that may discredit the concept established by over one thousand publications.

It is therefore of importance that three main criteria must be taken into account before using TF for clinical or animal studies: antigenic specificity and potency of the extract, and the immune status of the recipient.

II. Transfer factor as a preventative agent

The case for prevention

Probably, the most important potential for TF lies in its use for prevention, i.e. as a vaccine offering prophylaxis by specifically addressing cellular immunity. Several reports have shown that when a virus-specific TF is administered before an encounter with the virus, the recipient is protected. The most significant studies in this respect are those of Steele's team, who reported protection a) of marmosets from a lethal HSV-1 injection using TF from a human HSV-1-positive donor (Steele et al., 1976), and b) of leukemic children from varicella zoster virus using a VZV-specific TF (Steele et al., 1980), as well as those of Viza et al. (1986), who managed to protect mice against a lethal injection of HSV-1 or HSV-2. Additional clinical observations have confirmed the prophylactic activity of TF. Thus, several studies have described significant improvement using HSV-specific TF from human or bovine origin for the "treatment", i.e. prevention of relapses in patients suffering from recurrent genital and/or labial herpes, or recurrent herpes keratitis (Khan et al., 1981; Dwyer, 1983; Viza et al., 1983; Rosenfeld et al., 1984; Meduri et al., 1996; Pizza et al., 1996b).

As vaccination and/or treatment for several infectious diseases, viz. hepatitis C, AIDS, tuberculosis, are inexistent or at best unsatisfactory, and as conventional vaccines may sometimes generate serious side effects (e.g. Schoenfeld and Aron-Maor, 2000; Couette et al., 2009; Mayeta et al., 2011; Montastruc et al., 2011), the use of TF for prevention is of interest and warrants attentive consideration.

The black box effect

TF's "replicative properties" have not been fully exploited in producing standardized preparations with new specificities. Since the initiation of the clinical studies, antigen-specific TF has been mostly obtained from patients' household contacts. Results have been often, but not always, remarkable, reminding us inter alia that, because of genetic diversity, and unlike inbred animals, humans do not always respond in an identical manner to the same pathogen. Nevertheless, antigen-specific TF from household contacts is obviously of limited supply and cannot possibly benefit more than one patient for a limited period. Active TF, on the other hand, can be replicated in tissue culture (Viza et al., 1975, 1982, 1983) or be injected into animals to produce unlimited quantities of fresh TF (e.g. Boucheix et al., 1977; Viza et al., 1986), carrying the same specificity against the culprit microorganism that the donor's immune system has already recognized, even if the bug has not been identified by the laboratory (Viza et al., 1987).

This is what Viza has called the "black box effect" (in Pizza et al., 2006). There are two ways for exploiting it:

a) TF from patients recovering from infection by an unknown pathogen is replicated by animal injection or in tissue culture by a lymphoblastoid cell line; the tissue culture cells or the naïve animals function as an efficient copier, receiving and reproducing CMI information carried by the inducing TF molecules, whose target antigenic specificities are yet unknown.
b) Unidentified pathogens from patients' tissues are injected to experimental animals to produce an antigen-specific TF. This process was used for the preparation of the first HIV-specific TF in 1983, well before the viral aetiology of AIDS was established and the virus identified (Viza et al., 1987). Such procedures could be applied with similar success to other emerging diseases caused by identified or unidentified microorganisms, including manmade ones, as it is envisioned in scenarios of bioterrorism or in biotechnology laboratory accidents.

The threat of new pathogens

After the *Legionella bacterium*, Ebola, SARS, the new human herpes viruses and HIV are some of the new viruses that have emerged in half a century, in addition to the continuously emerging new strains of flu viruses, each time with the threat of a pandemic. The West Nile virus contaminations in the New York area (Weiss et al. 2001), and the Yosemite Park hantavirus cases (Hooper, 2001; WER, 2012) illustrate the unrelenting appearance of new infections. Moreover, the pathogenicity of the known microorganisms seems to be increasing.

For instance, Lyme's disease, the most common tick-borne disease caused by *Borrelia burgdorferi* (BB), produces in the majority of patients multiple co-infections generated by tick-borne mycoplasma, rickettsia and protozoa. Long-term antibiotic/antiprotozoan therapy is required, and restoration of the immune system necessary. It is worth mentioning here that the use of a BB-specific TF in infected patients has produced encouraging results (Viza et al., unpublished data). TF may thus be effective in addressing identified pathogens or new epidemics, especially when conventional vaccines are not available (Pizza et al., 2006).

HIV infection and AIDS

The observations cited here support the claim that TF could be used as an adjuvant treatment of the HIV infection and AIDS. Indeed, HIV's targets are lymphocytes mediating cellular immunity, and a DLE can stimulate and partially restore their number and activity as many observations suggest. For instance, restoration of DTH by IMREG, an antigen-non-specific component of the DLE, was observed (Gottlieb, 1991; Gottlieb et al., 1991), whereas lymphocyte extracts containing HIV-specific TF seem to help patients resist the syndrome (Carrey et al., 1987; Viza et al., 1987; McMeeking et al., 1990; Pizza et al., 1996a; Raise et al., 1996). This is consistent with several observations of patients spontaneously resisting the HIV infection. Indeed, as early as 1993, cases of patients strangely resistant to the viral infection were reported (Rowland-Jones et al., 1993; Langlade-Demoyen et al., 1994; Bryson et al., 1995). Such individuals frequently exposed to the virus did not develop an HIV infection leading to AIDS, but presented HIV-specific cytotoxic lymphocytes (Rowland-Jones et al., 1995), an observation consistent with that of Walker et al. (1986).

These initial observations, consistent with data showing that CMI and TF play a role in fighting viral infections, were pointed out to suggest that AIDS research should be focused on cellular rather than humoral immunity, the latter failing to produce a vaccine for nearly three decades, despite repeated promises and great expense (Viza, 1996). The evidence for the role of CMI in the control of AIDS is probably as overwhelming as the failure of humoral immunity to neutralize the virus. Thus, the use of HIV-specific TF as an adjuvant treatment for dealing with the HIV infection warrants investigation, and the hypothesis proposed some time ago, namely that resistance to HIV is dependent on T cytotoxic lymphocytes and that TF can instruct them to resist infection, is still pertinent (Viza, 1996).

Recent data support this thesis. Resistance to HIV depends on genes of the HLA complex that play a role in the immune recognition of the virus by the T lymphocytes, and the presentation of the viral capsid to the T cytotoxic lymphocytes is crucial in this process (Pereyra et al., 2010). These observations may be compared to that of Grakoui et al. (2003) showing that control of HCV viral replication depends on CD8 lymphocytes with the cooperation of CD4 lymphocytes. The absence of such cooperation results in the emergence of viral-escape mutations in class I major histocompatibility complex-restricted epitopes and in the failure to resolve the HCV infection. Furthermore, the evidence that CMI plays an essential role in the control of HIV infection supports the hypothesis that HIV-specific TF may be used to enhance resistance, i.e. as an HIV-specific vaccine addressing the CMI to develop prophylaxis.

Tuberculosis (TB)

In 2009, A. Fauci, Director of the NIAID, stated that *"the consequences of inattention to TB research are not just embarrassing, they are tragic and shameful*". The number of new cases is now estimated over 9 million, and the number of deaths 1–7 million each year. Drug-resistant strains of the bacterium have emerged but, with the exception of rifabutin, no new drugs have been developed in nearly half a century. The AIDS epidemic has exacerbated the problem, especially in developing countries; there are a great number of undiagnosed TB cases in HIV-infected patients. Early detection and treatment should curtail the spread of the bacillus and decrease opportunities for its transmission.

TF should be able to play a role by specifically stimulating the CMI. Indeed, immunocompetent individuals are able to eliminate or contain the bacterium in a latent state. Latent TB is an asymptomatic infection, without consequences for the bearer of living bacteria, as long as the immune system controls the replication of the bacillus, a situation reminding that of the HSV infection. It is estimated that two million people in the world have latent infection today (Hernandez-Pando et al., 2000), and it seems that CMI plays a crucial role in protecting these infected individuals from developing active disease by forming granulomas effective in containing the bacillus (Eley and Beatty, 2009; Paige and Bishai, 2010). Such granulomas are absent or poorly formed in immunocompromised hosts such as HIV-infected patients (Lawn et al., 2002; Bezuidenhout and Schneider, 2009). HIV infection impairs, numerically and functionally, *Mycobacterium tuberculosis*-specific CD4 lymphocytes and type-1 cytokine production, resulting in uncontrolled replication of the bacteria (Lawn et al., 2002). Vaccination is still the most obvious approach to this infection, and TF should play a role as several studies have suggested (Whitcomb and Rocklin, 1973; Rubinstein et al., 1977; Sharma et al., 1978; Fabre et al., 2004).

Flu viruses

Several new viruses with pandemic potential have been identified in the last 20 years, the most recent being the H1N1. Against such a pandemic, with a limited number of active antivirals to combat it, containment of the infection is unlikely, and the emergence of resistant strains is predictable. The preparation of new vaccines in a short period is an additional challenge (Kreijtz et al., 2009b). The Congressional Budget Office (CBO) estimated that *"a severe pandemic of avian flu could hit the*

United States hard, killing 2 million Americans and pushing the economy into a major recession". The agency underlined that such pandemics are unpredictable, and looking at episodes back to 1700, the odds of an influenza pandemic in any given year are about 3–4 %.

There were three global pandemics in the 20th century, and scientists have been repeatedly warning of a potential influenza pandemic comparable in magnitude to the 1918 Spanish flu. In 2003, avian influenza strain H5N1 gave just such a scare, and bureaucrats worldwide drafted one plan after another only to be prepared. However, the pandemic that erupted did not fit the theory.

A new H1N1 influenza strain emerged a few years later and obliged the WHO International Health Regulations (IHR) Emergency Committee, with the encouragement of vaccine producing laboratories, to declare a global pandemic emergency in 2009 that expired on August 2010; the virus did not prove to be as virulent as originally thought, nor the vaccine as harmless as initially claimed (Mayeta et al., 2011; Montastruc et al., 2011). Scientists have frequently speculated that this mild virus could mutate or exchange genes with other related viruses and become more aggressive, but in the end, the H1N1 will be remembered more for the panic it caused and the profligacy in producing large amounts of a useless and sometimes harmful vaccine. Nevertheless, an influenza epidemic will break out one day, and it may even be produced by the accidental escape of a laboratory engineered recombinant virus, but after so many false alarms, the public may have become weary. As discussed elsewhere (Pizza et al., 2006), the use of a specific TF for the prevention and treatment of new deadly flu viruses should be considered as an alternative to massive production of a new vaccine in a very short span of time. Meanwhile, the proposed theoretical approach should be corroborated by experimental data, and animal models are available to this end (Kreijtz et al., 2009a).

Perspectives and concluding remarks

In the years to come, prediction and prevention should become the pillars of medicine. Genomic scrutiny will help to build individual profiles, and tools for prevention will be engineered and adapted to specific pathogens, as well as to patients. TF should be in the arsenal of weapons for the prevention and treatment of known and newly emerging pathogens, as well as their syndromes. Inflammation, on the other hand, should also be fought by suppressing the infectious agent as well as by a direct action on its complex mechanisms by stimulating the activity of suppressor lymphocytes and regulating cytokine secretion. TF, i.e. the dialysable lymphocyte extract, is an immunomodulator with helper and suppressor activities, able to stimulate the suppressor lymphocyte subpopulation and modify the lymphokine secretion pattern (for instance: Kirkpatrick and Gallin, 1975; Lawrence and Borkowsky, 1983; Vich and Viza, 1983; Lawrence, 1995; Alvarez-Thull and Kirkpatrick, 1996; Gómez Vera et al., 2010). This might be of interest in syndromes such as Alzheimer's, where inflammation seems to be a major factor (Akiyama et al., 2000; Tuppo and Arias, 2005; Zotova et al., 2010).

As soon as the present uncertainty concerning its structure is resolved, TF should thus surface from its present quasi-oblivion and contribute to the battle for health. Its main advantages are: efficacy for treating and also for preventing infections, low manufacturing cost, and absence of toxicity. Indeed, the current paradoxical situation should not last. The overwhelming amount of data gathered over the last sixty years have not been contradicted or discredited by new studies, despite the existence of easy laboratory tests and inexpensive animal models. And biomedical research should be unconstrained by dogmatic considerations, never rejecting or ignoring unexplained or theoretically disturbing data. The principles, adumbrated by Bruce Alberts in his lectures as inherent to science, viz. rationality, creativity, openness, and tolerance, should serve as guidelines. And one should remember the recent statement of Ewan Birney, coordinator of the ENCODE consortium: "*I get this strong feeling that previously I was ignorant of my own ignorance, and now I understand my ignorance. But this is progress."* (Hall, 2012).

Letting observations fall into oblivion is only justified when the available technology is not adequate to pursue an investigation, and it does not warrant the cost or the effort for further exploring a hypothesis. But TF is not dark matter, and even less dark energy; it can be explored without waiting for an epiphany. The present advances in biotechnology should allow elucidation of TF's structure and mechanism of action at the molecular level. There has been some recent progress in this area. Using the microarray technology (Schena et al., 1996; Brown and Botstein, 1999) for instance, our preliminary data suggest that TFs carrying different antigenic specificities when incubated with the LDV/7 cell line modify the profile of its gene expression. The pattern of this polygenic effect seems to be linked to the antigen specificity of the inducing extract.

Currently, research is totally dependent on funding and a vicious circle is often created: lack of funding renders further studies impossible, particularly when interpretations are tinged with controversy. The solution may come from philanthropic institutions interested in solving health problems rather than governmental agencies encumbered by budgetary, bureaucratic, and political restraints, or pharmaceutical laboratories, which are not interested in studying compounds lacking patent potential. Tuberculosis and AIDS are real and urgent health problems, needing pragmatic solutions rather than losing time on theoretical arguments of byzantine complexity. In our opinion, transfer factor should play an important role in tackling infections.

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