Review Article
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Dialyzable leukocyte extract (transfer factor) as adjuvant immunotherapy in the treatment of cancer

Abstract

On 1955, Lawrence discovered that a dialyzed of viable leukocytes obtained from a healthy donor presenting a positive percutaneous tuberculin test was able to transfer to a healthy receptor the ability to respond to this pathogen. Lawrence named these molecules as Transfer Factor (TF). TF is composed of peptides obtained from lymphocytes ranging from 3500-6000kDa. T lymphocytes have the ability to express delayed-type hypersensitivity and cell mediated immunity from sensitized donors to noimmune recipients. TF plays a vital role in controlling immune over reactions and mistargeted responses in the development of autoimmune reactions. TF improves cellular immunity in patients with immune deficits due to their responsesare mediated by antigen-specific, inducer, and suppressor/regulatory activities contained in this fraction. Also, it has been observed that the mutagenic activity of whole TF and the suppressor activity to mutagen activation in TF is contained in Fraction I. Fraction II has a direct Chemiluminescence (CL) inductiveeffect on non-stimulated cells and increases the CL of phagocytes. Fraction III contains components responsible for the increases in the phytohemagglutinin (PHA) and pokeweed mutagen (PWM) responses. Antigen-specific fractions aid the function of recognizing and memorizing pathogenic organisms faster. TF also induces the antigenic stimulus whereas that the suppressor fraction acts by releasing IL-10, an inhibitory cytokine from Th2 cells. Therefore, TF could be considered as a new approach in the therapy of immune increasing in cancer

Keywords: transfer factor, immune response, cancer; immunotherapy, immuno modulatory

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Abbreviations: DLE, dialyzable leukocyte extract; CMI, cell-mediated immunity; TF, transfer factors; DTH, delayed-type hypersensitivity

Introduction

Dialyzable Leukocyte Extract (DLE) 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. Those products, also called Transfer Factors (TF), are peptides of 3500-6000kDa in molecular weight; light yellow fluid with pH 6.5-7.0, composed of oligoribonucleotides attached a peptide molecule that is inherent in all animal bodies. Due to TF has a small size it could act as an immunomodulator providing a cryophilic property. TF is thermolabile but has a high stability under cold temperatures. TF can be preserved foryears between -20°C and -70°C.

In 1955, Lawrence showed that TF carrying a donor's antigenic specificity couldbe retrieved from the lymphocytes of a naïve recipient previously injected with the donor's TF. The lymphocytes of the recipient subject act as an efficient copier, integrating the specificity of the injected TF, and the TF recipient is thus becoming an effective donor. Lawrence assumed that this adoptive transfer of immunity was due to the molecules less than 12kDa.[1,2] After that, Lawrence did new experiments using ligates of blood leukocytes from donors who had positive delayed-hypersensitivity to antigens such as tuberculin (PPD), diphtheria toxoid, streptococcal M protein, or coccidioidin. The recipients were initially skin test-negative to the test antigens. Within hours after receiving the transfer factor, the recipients were able to express delayed hypersensitivity to antigens to which the donors were reactive.[3]

TF have high levels of tyrosine and lysine residues with a significant similarity to the N-terminal regions of some neuropeptides, such as those of the encephalin family.[4] In general, all TF tested were very efficient factors to correct or enhance CMI responses besides to show an antigen-specific response.[5–7] On 2000, Kirkpatrick obtained particular TF by immunizing mice and cows with ovalbumin, Herpes simplex glycoprotein D, and transferring. He was able to purify specific TF for each of these antigens, having the ability to transfer a cellular response in a specific way evaluated by the cell-mediated immunity or delayed-type hypersensitivity (DTH) when challenged with these antigens. When the sequences ofpeptides were analyzed, it was found a consensus sequence MXLLYADQDL/VEDN. However, when the biological effect of this synthetic sequence was tested, it was not able to transfer the specific response type DTH, but it blocked the transference of specific TF, which suggested that this sequence binds to the TF receptor.[8]

Obtaining of TF

The extract of TF has several biologic activities. Some fractions of TF have been separated by Low-pressure liquid chromatography, electrophoresis, and enzymatic methods.[9–11] Briefly, blood is collected from people whose previously had contact with the disease. This blood was drawn into an Ion Exchange column. The supernatant plasma and leukocytes were removed with a plasma extractor into a transfer pack and centrifuged in the pack, and the cells were lysed in sterile conditions. The transfer factor was recovered by dia filtration

and concentrated by tangential ultrafiltration.[12] Finally, TF was obtained when the leukocytes were thawed at room temperature and the dialysate was lyophilized. Since TF has not yet been characterized chemically, the preparations are assayed for total solids, ash, biuret-positive material, and nitrogen, and subjected to spectrophotometric analysis to provide some indication of uniformity. Through gel diffusion against antibody directed against whole human serum, it was ascertained that preparation was no contained plasma proteins. The dialysis was passed through a pore size 22µm filter and stored frozen in sterile vials until it will be used.

Some studies were development to recognize the fraction that exerts its effects on cells. By modifying the Sephadex G-25 chromatographic conditions two fractions, IIIa and IIlb were separated. *In vivo* activities of these two fractions indicate that only IIIa fraction obtained from positive antigen donors were able to transfer positive skin reactivity. While, fraction IIlb alone produced substantial antigen-independent skin reactivity.[13] On the other hand, when TF was applied to SephadexG-10 separation columns, two components were obtained named as "L" and "S" fractions. L was the not dialyzable sub-fraction, and the S fraction contained 85% of the total of polypeptides including the antigen-independent inflammatory component. When this fractionwas administrated in patients, it induced anintradermalreaction, and per vascular infiltrates of mononuclear cells around the blood vessels.[14] This study determined the efficiency of MTT assay for detection of bactericidal or bacteriostatic effects of bDLE and "S," as confirmed by other authors.[15] In this study, the antibacterial effects of "L", I, II, III, and IV fractions were not detected probably due the presence of other substances that inhibit the bacterial growth, the lack of combinations tested among the I, II, III, and IV fractions, or both situations. These results showed a remarkable in vitro antibacterial property of bDLE against several pathogenic bacteria independent of the immune system and its potential as an antibacterial agent may be confirmed with further *in vivo* studies.[16]

The mutagenic activity of whole TF and the suppressive activity to mutagen activation when present in TF was found in Fraction I. Fraction III contained components responsible for augmentation of PHA and PWM responses. Additionally, in Fraction III was found the component responsible for the increase in the antigen-dependent of lymphocyte transformation. Fraction IV was suppressive to antigen-induced lymphocyte transformation. They found only fraction II having a direct CL inductiveeffect on the non-stimulated cells. Fraction II increased further the CL of phagocytes which were stimulated by zymosan (Mannozym). Fractions I and III were ineffective, suggesting that the stimulation of the peripheral blood phagocytes by the oligopeptides of fraction II may have an important role. The studies for determining the effects of dialyzable leukocyte extracts (as it is reflected in the recovery of E-rosetting capacity in trypsinized lymphocytes), has allowed proposing the following: Dialyzable leukocyte extracts contain an inhibitor with a molecular weight of about 5000 (fraction I). This inhibitor considerably affects the immunologic activity of both whole preparations and their different fractions during the *in vitro* activity assay. Immunological activity is concentrated in fractions III and IV. Only fraction III activity can be reduced considerably by inactivation. Fraction II activity is induced by incomplete separation from fraction III. Meanwhile, Fraction IV cannot be inactivated.[17]

The purification of TF by chromatography was achieved by fractionation of a hypersensitive cell extract. The cell supernatants were first summited to chromatography assayson Sephadex G-200 columns. Increasing the bed height caused a change in the number as well as in the composition of the eluted fractions as it was determined by immuno electrophoresis, agar gel diffusion, and ribose and N concentration. The composition of each fraction was related to the ability to transfer hypersensitivity to recipients. The transfer capacity was always found in two fractions.[10] The C-fraction, which transfers delayed hypersensitivity, upon further purification yielded the C'-5 polynucleotide which was an active transfer agent. The C'-5 polynucleotide is of interest due it contains ribose, adenine, guanine, and cytosine, but has no uracil. No amino acids were detected in this fraction. On immuno electrophoresis or agar gel diffusion, no precipitated bands were found when tested with antisera against human serum, human white blood cells, or purified human, G-globulin. Furthermore, the transfer capacity residing in the C'-5 polynucleotide was also found in other cell fractions eluted from the Sephadex G-200 columns. The composition of these fractions also changed as the column height was increased. These three fractions all contained 5, G-globulin and protein which upon immuno electrophoresis migrated in the serum to lipoprotein zone. Some other precipitin bands were also present. However, it is important to note that ribose was also present in each of these fractions, probably indicating RNA.[10]

The A fraction from the 12- by 200-mm column contained macroglobulins and ribose. It is of interest to note that this fraction did not transfer hypersensitivity. This fact would indicate that the macroglobulins are not involved in the hypersensitivity nor is the RNA in this fraction, indicated by the presence of ribose, capable of inducing delayed hypersensitivity in recipients. On the basis ofthe observed N concentration and the capacity to transfer hypersensitivity, the A' fraction may probably be considered purer than the B and the A fractions. The ultra centrifugal analysis of the A' fraction showed two peaks. One of these comprised over 90% of the sample and had an s20, compatible with 7G globulin. However, this fraction has been shown to have many components on immuno electrophoresis. The transfer of tuberculin delayed hypersensitivity with disrupted white blood cells has been found to persist for at least two years. These data could indicate either the passive transfer of an unusually stable antibody, which is unlikely or, more probably, the induction of active hypersensitivity in the recipient. Both the dialyzable and non-dialyzable fraction has been shown previously to produce sensitivity of long duration in recipients. It is possible that antibody may be present in the A' fraction which could passively sensitize recipients. Because of the long length of the actively sensitizing transfer factor in this fraction, any antibody present causing passive sensitization would be masked. Transfer of tuberculin delayed hypersensitivity must be attempted with purified globulin before the possibility of the existence of antibody in the A' fraction can be eliminated. The capacity of the two transfer factors to induce long-lasting delayed hypersensitivity in recipients may be explained by two facts:1) RNA or polynucleotide carrying specific information to immunocompetent cells, 2) RNA or polynucleotide-acting as a carrier for antigen, or 3) antigen present in a form which in low concentration can induce delayed hypersensitivity in cells.[10] Nowadays, TF can also be obtained either from colostrums of cow, goat or even from chicken eggs due to its low cost.

Mechanism of action

T-lymphocytes produce transfer factors, and they can transfer the ability to recognize a pathogen to cells which have not been in contact with the pathogen yet. They also heighten the immune system ability

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to increased reactivity or induce function to pathogens. TF may act as a gene product that assists in antigen presentation to other T-cells. This inducer fraction of transfer factor links the immune cells with an antigen-binding site, thereby increasing their reactivity to an antigenic stimulus. The suppressor fraction blocks the response of the T-cells and signals a down-regulation of the immune response.

Transfer factor has bio feedback mechanism by antigen-specific, inducer, suppressor/regulatory fraction contain in it. Antigen-specificfractions aid the function of recognizing and memorizing pathogenic organisms faster. This hypothesis is sustained by experiments in isolated peripheral blood mononuclear cells and monocytes, where TF treatment displayed similar cytokine/chemokine inhibition and activation profiles for each of the bacterial cell wall components tested. TF exerts a specific and potent inhibitory effect on TNFα production by leukocytes and isolated monocytes stimulated by both Gram-negative and Gram-positive bacteria cell wall constituents. On the opposite, both IL-6 and IL-8 secretion by bacterial components-activated monocytes were elevated by TF. The ability of transfer factor to enhance IL-8 secretion supports the previous conclusion that TF contains potent chemotactic activity for granulocytes and monocytes.[18] The up regulation of the IL-6 and IL-8 production by TF might promote the resolution of inflammation by initiating neutrophils recruitment and favoring monocytes infiltration. TF potently inhibited the TNFα production in bacterial components-stimulated leukocytes, which are consistent with the observed high suppression by TF of the NF-kB binding activity in activated leukocytes. Some data suggest that TF could regulate TNFα secretion through the inhibition of the NF-kB activity (REF). Also, the increase of cAMP induced by DLE could be related to the DLE effect on pro inflammatory cytokine production. The pattern of TNFa, IL-6 and IL-8 release by TF appeared to be similar to that caused by intracellular cAMP increase in leukocytes (REF)

TF is a modulator of the immune response capable of transferring specific immunity to naive T cells.[12] That also inhibits the production of TNFα by monocytes.[19] TF has been reported to be effective in various pathologies caused by viral, parasitic, and fungal and mycobacteria infections. This evidence suggested that TF could modulate the response to the recognition of Gram-negative bacteria via activation of the Toll-like receptor 4 (TLR4)-MD2 complex which occurs through the MyD88-mediated NF-kB pathway.[20] Also, this is supported by our previous result that TF inhibits NF-kB activity in a human T cell line.[21] In spite of accumulated experimental evidence, the mechanisms that underlie the immuno modulatory effects of DLE have not been completely elucidated.[22]

In vitro models

TF has shown notable effects in *in vitro* model improving cellular immunity.[23] and regulating the production of different cytokines involved in tumor progression.[24–26] In an *in vitro* study, the authors described that TF has the property of negatively regulate the production for the mRNA of osteopontin, a cytokine with a regulatory effect in the immune system. The IFN-Gamma is a pro inflammatory cytokine that activates diverse cellular populations such as macrophages, neutrophils, Blymphocytes, NK Cells, and particularly the differentiation of helper lymphocytes into Th1 lymphocytes.[27]

Some reports have shown an antibacterial effect of DLE, due to the transfer factor specific to the immune system–dependent antigen present. In a study, Amides et al demonstrated the direct effect of bDLE on bacteria.[28] Is independent of the immune system and its antigens. The data showed that bDLE and its "S" fraction significantly affect the bacterial growth. Some researchers believe that DLE has antigen-dependent specific effects, while some others believe that its effects are nonspecific. However, some studies indicate that both, antigen-dependent specific and nonspecific effects are present in DLE.[29] The bacteriostatic and bactericidal effects of bDLE and its "S" fraction are dose-dependent, as well as dependent on the particular bacteria and its concentration. These effects may alsobe influenced by the presence of cyclic nucleotides, prostaglandins, serotonin, histamine, ascorbate, nicotinamide, some amino acids, and purines that are found in the DLE.[30]

Another assay made in breast cancer cell line assays, bovine TF (bTF) induced cytotoxic effects by suppressing the expression of p53 mRNA, bab-1, c-myc, bax, bcl-2 and bad mRNA which have effects on apoptotic processes in these cells.[16,31]

Animal models

Kirkpatrick demonstrated that *in vivo* administration of TF to mice, afford the recipient's spleen cells with the property of responding to target antigen *in vitro* by secreting IFN-γ, a product of Th1 cells, IL-2, and TNF- α, thereby ensuring the development of cell-mediated immunity. While TF stimulated cell-mediated immunity, it was not observed any decrease in the antibody secretion or in the responses against the same specific antigen.[32] The findings of specific interactions between TF and antigens provide some insight into the structural basis of specificity. Furthermore, the finding that TF reacts with native antigens suggests that it is not a dialyzable fraction defined by T cell receptor for antigen.[33]

In Glioma C6 model; TF, carmustine, and both TF and carmustine were administered and compared.[34] The combination of TF with carmustine provided the best results, followed by the administration of TF alone. It was observed a minor improvement with the administration of carmustine alone. Additionally, animals treated with TF showed an increase of lymphocytes T CD4+, T CD8+, and NK cells. Furthermore, there was an increase in the production of IL-2, IL-6, and TNF-α, which is a remarkable finding considering that it has been reported a decrease of IL-2 in patients suffering gliomas. Recent studies in murine recipients have shown that *in vivo* administration of TF increasethe capability of the recipients' spleen cells to responding to the corresponding antigen *in vitro* by secreting IFN-γ.

Cancer and transfer factors

Since cancer has been associated with a TH1-deficient state, the clinical use of TF should be considered as an adjuvant treatment in cancer therapy.[35] TF has been shown to improve cellular immunity in patients with immune deficits and even increase the quality life of patients during chemotherapy.[36] It is well known that chemotherapeutic drugs produce T-cell depletion, which is more severe in CD4+ than in CD8+ T lymphocytes. Also, chemotherapy induces a decrease in the dendritic cell function and an altered production of pro-inflammatory and anti-inflammatory cytokines. Therefore, TF represents an attractive alternative to complement chemotherapy, which can be used to enhance the immune system after disturbances resulting from the side effects of chemotherapy. Here, we describe some examples of how the TF can be used as adjuvant therapy against cancer.

The use of TF in cancer has been described since the 70's.Espanol et al treated children with neublastomas by using lymphocytes of their

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mothers, observing the cytotoxic ability of these cells against the tumor cells.[36] In another assay made in the 70´s, the dialysate of an extract of leucocytes was administrated to 12bronchopulmonary cancer patients for 6days; Hainaut el al observed an increase of T- lymphocytes in almost all patients, besides a rapid healing of a widespread inter current zone and a region of B.C.G and a scarification reaction.[37]

Patients with estrogenic sarcoma received injections of estrogenic sarcoma-specific dialyzable transfer factor derived from healthy donors, and they found an increase of lymphocytes after administration of tumor-specific transfer factor in all patients so treated.[38] Also, Fudenberg showed that transfer factor could, from selected donors, increase the cell-mediated responses to tumor-associated antigens in human osteogenic sarcoma patients.[39] In this sense, patients with renal cell carcinoma were treated with transfer factor as an immuno modulator. These patients showed a temporary stabilization of metastatic disease without clinical regression of measurable disease.[40]

In malignant melanoma, the TF was used in 36 patients founding an increase in the survival of the groups treated. However, they did not observe an adjuvant effect of TF.[41]Tumor-bearing patients experienced pain and edema around the site as the tumor increased but these symptoms diminished concomitantly with the TF injections. Biopsy specimens taken from various tumors before and after TF therapy demonstrated the induction of antitumor immunity, as it was evidenced by lymphocytic and monocytes infiltration of tumor tissue.[42] Although some notorious results havebeenreported, the prospective trials evaluating the potential use of TF are scarce and still under investigation. Therefore, it would be desirable to initiate more studies concerning the knowledge of this complex of molecules to propose to TF as an innovative approach for the treatment of cancer patients.

TF has also benn used against melanoma. In 1980, 64 patients with melanoma, a highly- resistent-radio and chemotherapy tumor received a combined therapy of melfalan and decarbazin plus 1 unit of TF (equivalent to $10X10^9$ lymphocites) for 21days. Patients who received the TF had a remision of only 20percent with an increase in the survival of 4months in contrast with those who did not received the TF.[43]

In a prospective randomized, double-blind study, patients with invasive cervical cancer were treated with TF derived from leukocytes of the patients' husbands, and the other received placebo. Within the first twoyears after radical hysterectomy, 5 of TF-treated and 11 placebo-treated patients developed recurrence of malignancy. Immune profiles were checked in leukocyte donor's beforeleukophoresis and serially tested in patients. Antigen-specific correlations were found between donors' and recipients're activities but not between donors' reactivity and recipient's course of the disease.[44]

In clinical trials, patients with advanced breast cancer were treated with pooled dialyzable transfer factor from healthy adult donors without chemotherapy or radiotherapy,[45] after which the disease progressed(Figure 1-3).[46]

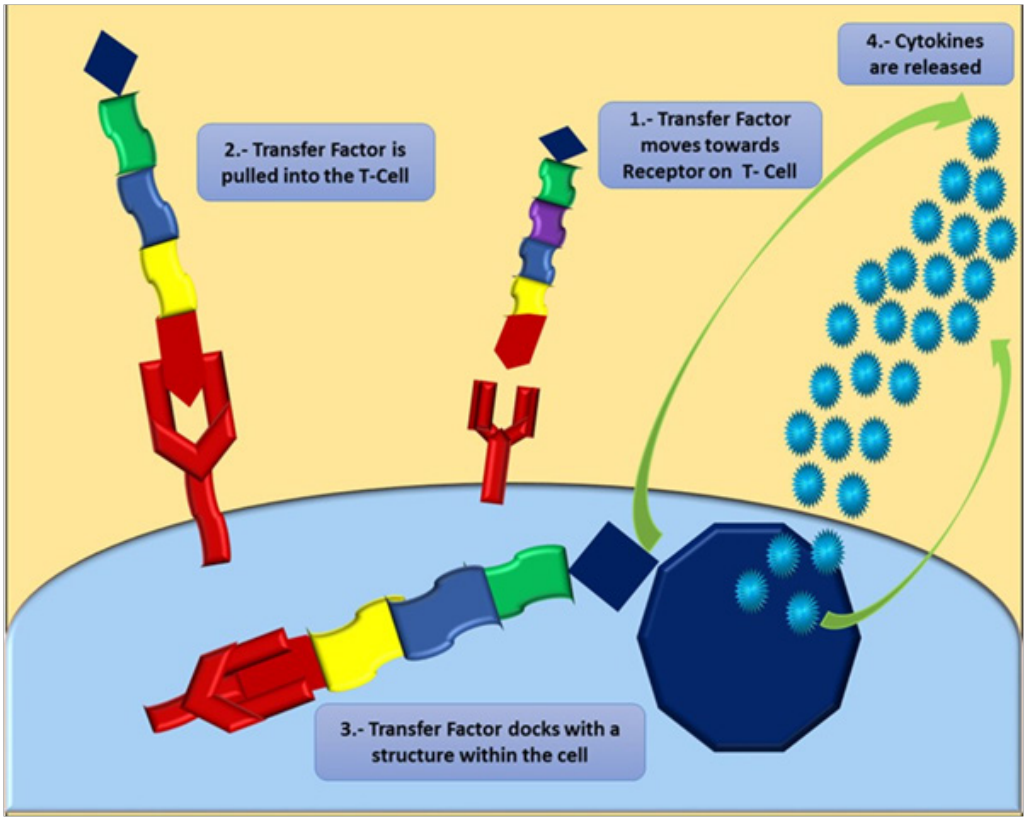

Figure 1 The structural form of transfer factor.

TF is a protein complex that inducesan immune response. TF is used as an adjuvant or as a molecule in the treatment of several diseases.

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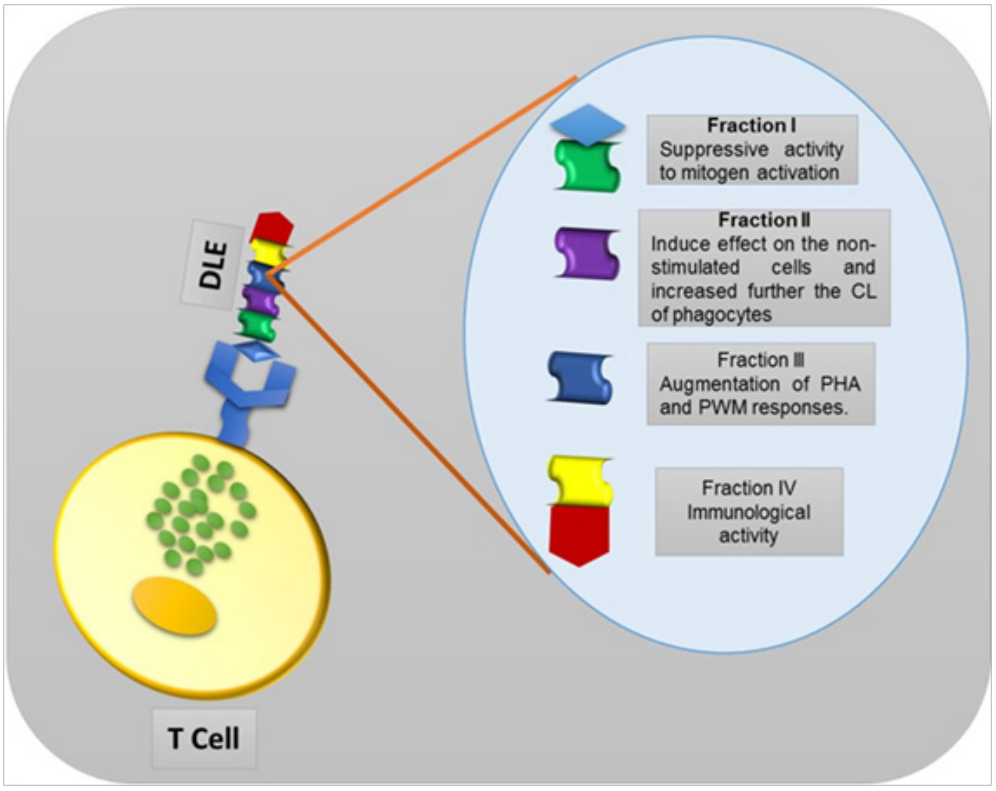

Figure 2 Purification of transfer factor fractions.
The biologically active fraction of human transfer factor has been separated by exclusion chromatography in some component fractions.

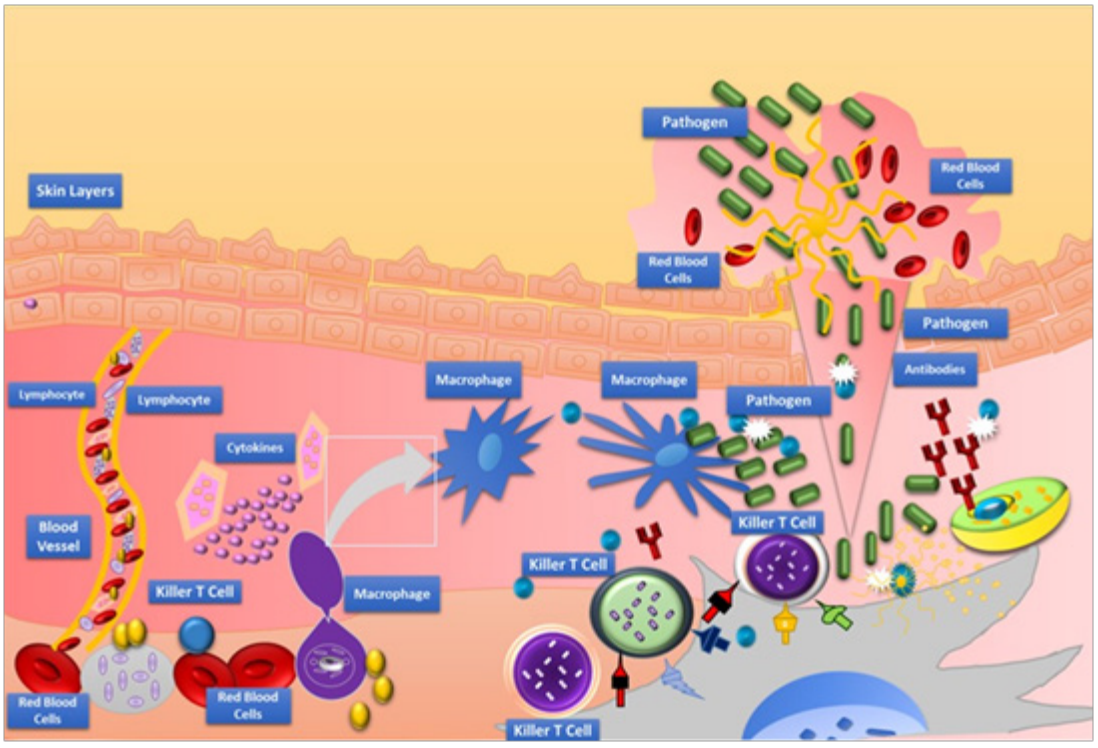

Figure 3 Transfer factor pathways.
Phagocytes present antigens and cytokines to CD4+ cells, which releases transfer factor antigen. Th1 cytokines give CD8+ killer T cells to seek and destroy tagger body cells. Transfer factor helps to improve the immune response.

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Conclusion and perspectives

Despite the fact that TFs were discovered over 60years ago and their vital role in control of immune reactions, very few in vitro studies and clinical trials have been made in order to test its potential activity in a Cancer treatment. Given its ability to recognize a pathogen to cells which have not been in contact with the pathogen yet, it is safe to assume these factors can be a viable immunotherapy in the treatment of cancer, either being a modulator of the immune response capable of transferring specific immunity to naive T cells that have not been in contact with the tumor cells or enhancing the immune system after disturbances resulting from the side effects of chemotherapy, qualities that make the TF a subject worthy of interest in the search of alternative therapies against cancer.

Acknowledgments

None.

Conflicts of interest

Author declares that there is no conflicts of interest.

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