Chapter 14

Immunotherapy with Dialyzable Leukocyte Extracts Containing Transfer Factor

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66524

Abstract

Dialyzable leukocyte extracts (DLE) are complexes consisting of a large number of low molecular weight substances. These extracts possess immunomodulatory properties, which are mainly attributed to small peptides with molecular weight of 3.5–6.0 kDa called "transfer factor." This chapter reviews the nature and immunological characteristics of DLE containing transfer factor (TF), their mechanism of action and the possible uses as immunomodulators in human and veterinary medicine. A main advantage of TF-preparations as immunotherapeutic agents is that they induce a rapid immune response against the pathogen (within 24 h) and thereby reduce the time for the patient immune response by 9–13 days. The low level of difficulty of the process of obtaining protocols determines their relatively low cost and the possibility to combine them with other therapeutic agents during treatment makes them subject to medical applications in the future, including against some new diseases.

Keywords: animal models, cytokines, chromatography, diseases, dialyzable leukocyte extract, lymphocytes, transfer factor, ultrafiltration

1. Introduction

The immune system is extremely effective when it protects the organism from foreign (or own, but modified) genetic substances. However, some antigens that trigger the immune system—microbial or cancer cells, have devised a number of techniques to avoid the immune reaction. This means that the immune system constantly adapts to new attacks and eliminates the antigens by identifying each cell.

Autoimmune diseases occur when the recognition process of cells breaks down, resulting in the destruction of healthy cells and tissues. That is, when the immune system is essentially "popular up," which can be difficult to treat.

This requires the search for new approaches to immunotherapy of infectious, oncological and autoimmune diseases. In the recent years, preparations, called "dialyzable leukocyte extracts" (DLE), were introduced to immunotherapy.

The dialyzable leukocyte extracts are complexes that are built up by approximately 200 substances that have low molecular weight [1–3]. DLE have immunomodulatory properties, which are due to small peptides—the "transfer factor" (TF). The rest of the components in the extracts have different biochemical and immunological properties and contribute to the immunomodulatory effect carried by TF.

In 1955, Dr. H. Sherwood Lawrence defined the transfer factor stating that cell-mediated immune response to antigens (allergens) can be passively transferred by DLE of viable human leucocytes from a donor of immunity to a naïve recipient [4]. He prepared an intracellular extract from circulating leucocytes of patients who had been exposed to tuberculosis (TB) and then injected the leukocyte extract into non-exposed to TB volunteer patients. Using a delayed type hypersensitivity test, Dr. Lawrence demonstrated that the immune system of non-exposed to TB patients treated with leukocyte extract can recognize TB and response to it, as if has already fought it. Therefore, the immune response to a certain antigen can be "transferred" from one person to another using a leukocyte extract. The transfer factor can instruct the immune system to do several different things, thereby influencing immunity via different paths and can come in three different sizes. Thus, the induction of a number of different effects on the immune system released by leukocyte extracts implies the presence of more than one active factor in it, and there seem to be multiple factors involved in transferring immunity [5]. One activity is the presence of the inducer and helper functions (the so-called inducer factor and antigen-specific factor). An additional activity is the presence of the suppressory (regulatory) function (the so-called suppressor factor) [6].

Later on, it was found that the transfer of immunity by leukocyte derivatives is also possible from one species to another. This universal ability makes it possible to get the TF-preparations from a species which is different to the recipient species.

The discovery of this phenomenon gave new opportunities in the field of medicine. Since cell-mediated immunity (CMI) is crucial for controlling infections, as well as cancer, autoimmune diseases, immunodeficiencies and allergies, the transfer factor can be used in the treatment of these cases [1, 3, 7–10].

The author will review the nature and immunological characteristics of DLE-containing TF, their mechanism of action and the possible uses as immunomodulators in human and veterinary medicine.

2. Composition, physicochemical and biochemical properties of DLE

Transfer factor, the main component of the DLE, has a molecular weight of 3.5–6.0 kDa. It consists of small peptides and oligoribonucleotides [3]. The ribonucleotide is attached to the amino terminus of the peptide. The specific activity of TF is due to the peptides weighing 5.0 kDa [11].

Transfer factor peptides have the same amino acid sequence, regardless of their origin and species belonging. In 2000, while analyzing the peptide partial sequences of transfer factors, Kirkpatrick [12] found a novel amino acid consensus sequence LLYAQDL/VEDN, which is found in each of the analyzed TF-preparations. This novel sequence binds with high affinity to specific receptors of target cells (the so-called TF receptors). However, tyrosine and glycine are always more concentrated in TF [9]. The N-terminal region of these peptides is very similar to some neuropeptides, such as the enkephalins [13].

Apart from transfer factor, the extracts also contain cyclic nucleotides, ascorbate, prostaglandins, histamine, serotonin, nicotinamide and some amino acids and purine bases [14]. DLE preparations are transparent, pyrogen-free, light yellow fluids, with pH 5.5–7.0 or lower (pH 5.6–6.8) depending on the method of obtaining [2, 10, 15]. The ratio OD_{260}/OD_{280} (absorbance index, which represents the ratio of nucleotides to peptides) of the extracts ranges from 1.8 to 3.0 depending on the method of preparation [15–18]. The index values show that nucleotides are relatively predominant over peptides. The osmolarity of DLE preparation was measured by Grob et al. [15] and has a value of 520 ± 90 mOsm/L.

TF is resistant to treatment with DNase, pancreatic RNase and trypsin, but cannot withstand snake venom phosphodiesterase. It is stable in conditions of deep freezing, but it is not over certain temperatures. In particular, DLE preparations are biologically active for several years when kept at temperatures ranging between −20°C and −70°C. The fact that the double-stranded nucleic acid can melt determines TF's heat sensitivity [10]. As it is a low molecular weight mixture, the dialyzable leukocyte extracts are not immunogenic and contain no histocompatibility antigens [3, 10].

3. Mechanism of action and immunological properties of the transfer factor

The transfer factor is produced by CD4+Th1 cells during the immune response to an antigen. Its biological activity includes different, sometimes opposite effects. Transfer factor contains the following constituents: inducer, antigen-specific and suppressor factors [6, 10].

The inducer factor sets the immune system in a state of readiness by sending a specific signal to the cells. When nonimmune leukocyte populations are cultured with inducer factor, they acquire the capacity to respond to a specific antigen [6]. It enhances the antigenic stimulus, which causes the production of interferon gamma (IFN-γ), interleukin (IL)-2 and tumor necrosis factor alpha (TNF-α) by CD4+Th1 cells. As a consequence, cell-mediated immune response develops against the target antigen [10] and it includes interleukins (Il-6 and Il-8) formed by activated monocytes [19, 20].

The regulation of the production of TNF-α, Il-6 and Il-8 is associated with effects on toll-like receptors (TLR2 and TLR4) expression and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclic adenosine monophosphate activities [19]. Subsequently, however, the role of the ligands for TLR4 as regulators of the production of these cytokines was excluded [21, 22].

The antigen-specific components help the immune system to identify the antigens by using a variety of tags that identify them. They are informational molecules that are involved in the immune recognition of antigens that have penetrated the organisms and are also included in the formation of the immune memory.

When immune leukocyte populations are cultured with suppressor factor, their response to a specific antigen is blocked [6]. The suppressor factor maintains the balance in the immune system, preventing its overactivity in the absence of any new threats to the body. This helps to control the autoimmune diseases and to improve the adaptability of the immune system. In general, the mechanism of action of the suppressor factor can be defined as a catalytic immune response. Suppressor fractions are involved in the regulation and weakening of the immune response to the antigen by stimulating the formation of IL-10 and inhibiting cytokines by Th2 cells [6, 10, 23]. According to Burger et al. [23], a component of TF (fraction IV from exclusion chromatography on Sephadex G-25) possess immunosuppressive activity. This component was identified as nicotinamide. In our study on the immunological activity of rabbit DLE, we found that suppressor ingredients of the extract have different molecular masses (in peaks I, II, III, V and VI)—**Figure 1**.

In the dialyzable leukocyte extracts, molecules with adjuvant-like activity can also be identified. This component of DLE possesses a nonspecific activity expressed by enhancing the immune response to other antigens or allergens [24]. These fractions are with molecular weight <3.5 kDa and cause two types of immune response: (1) amplification of the response to antigens to which the donor has preexisting immunity and (2) the induction of inflammatory response histologically resembling delayed hypersensitivity in the absence of an added antigen. The substances mediating these responses could be divided into unique components by the use of a long (1 × 150 cm) G-10 column or by hydroxylapatite chromatography. We found fractions with adjuvant-like activity in rabbit DLE in peak IV in gel filtration Sephadex G-25 (**Figure 1**) [25].

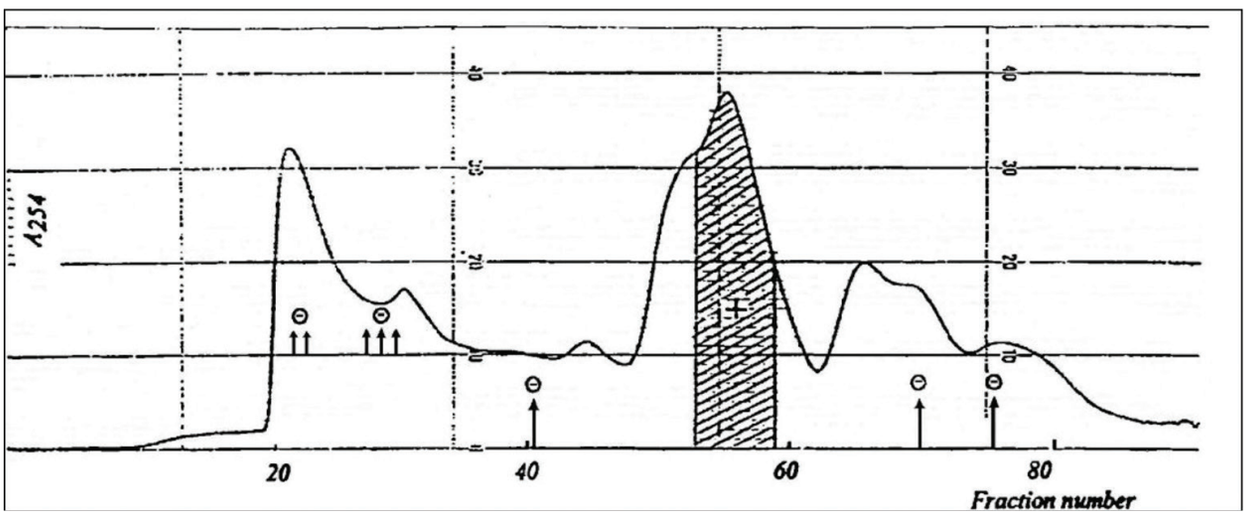

Figure 1. Elution profile of DLE, obtained from rabbit lymph nodes and spleen after gel-filtration through Sephadex G-25 (column 2.6/70). Liquid chromatography system Pharmacia. The fraction with adjuvant-like activity is marked with ⊕ and suppressor fractions are marked with ⊖ [25].

Fractions with hematopoietic activity can also be detected in the extracts [26] as well as with in vitro antibacterial activity [27].

In vivo, DLE enhanced recovery of the pool of granulocyte-macrophage hemopoietic progenitor cells (GM-CFC) in the bone marrow of normal or sublethally irradiated mice and increased survival of mice exposed to a lethal radiation dose. In vitro, sera of mice treated with DLE-induced GM-CFC colony formation in cultures of normal mouse bone marrow cells, i.e., produced colony-stimulating activity (CSA) [26].

Low molecular weight fraction of bovine DLE (below 3.5 kDa, the so-called fraction S) has bactericidal and bacteriostatic activity in vitro against pathogenic bacterial strains *Staphylococcus aureus*, *Streptococcus pyogenes*, *Lysteria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. These results showed a remarkable in vitro antibacterial property of bovine DLE against several pathogenic bacteria [27].

DLE regulate the expression of the hBD-2 and LL-37 genes [28]. Since the two peptides (hBD-2 and LL-37) have antibacterial action, DLE play a major role in the innate immune defense against invasive bacterial infections and inflammations. The probability that a microorganism shows resistance to these peptides is much lower than presented by antibiotics. However, these peptides have the ability to immunoregulate the acquired immune response, thus promotes inflammation without an eye injury.

Furthermore, the lymphocytes of a naïve recipient can serve as a replicator of transfer factor. This is achieved by integrating the specifics of the injected TF in the recipient lymphocytes, which effectively means that the recipient can be seen as a TF donor as well. This phenomenon is known as "the black box effect" and allows us to obtain TF-preparations from donors which have been infected with an unknown pathogen [29].

Therefore, TF develops CMI in patients who are suffering from immunodeficient infectious diseases, as well as in disorder with certain anergies. It is agreed that transfer factor is more efficient in educating naïve cells about the approaching danger [10]. It takes its part in the whole process of activation of the immune response by controlling and preventing immune overreaction and mistargeted reaction in the development of autoimmune diseases.

4. Obtaining transfer factor preparations

TF-preparations may be obtained from animal and human sources by injecting them with certain pathogen to produce specific transfer factor [10]. The first TF-preparations were obtained in the laboratory of Dr. Lawrence [4] though dialysis of human leukocyte cryolysates. Furthermore, dialysis as a separation method of low-molecular-weight components was replaced by ultrafiltration [8, 26, 30]. Membrane filters with a cutoff of <12 kDa are used in the procedure. Apart from human leucocytes, TF-preparations are obtained from ultrafiltrated animal cryolysed leukocytes or lymphoid organs (lymph nodes and spleen) [17, 18, 26, 31, 32].

Transfer factor can also be obtained from bird eggs and the method is patented [33]. TF with activity against HBV was extracted from egg yolk [34].

Another source of TF is bovine colostrum. A method for extracting TF from colostrum was prepared, which currently has very wide application [35]—the reason is that by using a relatively simple procedure, large amounts of TF can be obtained and the donor cows can be immunized with different antigens depending on the purpose. TF which has been obtained from bovine colostrum is patented as a commercial product (4life TF) [36].

DLE may be further subjected to purification using column chromatography, high-performance liquid chromatography [10, 37] and molecular exclusion liquid chromatography [38]. Through these procedures not only a better purification of the preparations can be achieved, but also different fractions can be isolated. The figure below presents the elution profile of DLE after gel-filtration on Sephadex G-25 (**Figure 1**).

Six peaks were obtained as the active fractions were eluted in peak IV. Some fractions in peaks I, II, III, V and VI possess suppressive activity.

5. Application of DLE preparations in human and veterinary medicine

The application of DLE or TF preparations in medicine is based on the influence of transfer factor and other components of dialyzable leukocyte extracts on the function of a number of components of the immune system and on regulating the synthesis of cytokine. When the immune system of an organism interacts with a pathogen, at least one TF is created in every instant and this reaction applies to all pathogens [39].

The rapidity of the reaction against pathogens is a strong advantage of TF-preparations as therapeutic agents (the response is within 24 h). This is a fraction of the time for a complete cell-mediated response of the immune system to a pathogen, which is 10–14 days.

Cancer, heart diseases, Alzheimer's, rheumatoid arthritis, hepatitis and other major diseases are caused by abnormalities in the formation of the transfer factor in CD4+Th1 cells. This determines the wide range of possibilities to use TF preparations in the field of medicine.

Another possibility of immunotherapy with DLE and TF is the use of the so-called the black box effect. There are two ways to use this effect: (a) initially receiving TF from patients recovering from infection with an unknown pathogen that replicate to naïve experimental animals or in tissue culture of lymphoblastoid cell line. The immune system of the experimental animals and lymphoblastoid cells from the tissue culture function as an effective copier, which can produce specific transfer factor activity to the unknown pathogen; (b) unidentified pathogen isolated from the tissues of the patient is injected into a naïve experimental animals that produce antigen-specific TF. This method has been used for the preparation of the first human immunodeficiency virus (HIV)-specific preparation TF in1983 before it is established viral etiology of AIDS [40].

In our studies in the 1990s, we have received and tested dialyzable leukocyte extracts of blood and lymphoid organs of pigs from farms with considerable respiratory and gastrointestinal diseases in newborn and young animals. Given the polyetiological character of these diseases, the approach of obtaining DLE through the "black box effect" proved successful—mortality and morbidity of these diseases on the farm that was treated with DLE were significantly reduced.

5.1. Cancer

There are about 100 reports on the effect of TF on cancer, either in patients suffering from cancer, or on animal and in vitro models.

Pineda et al. [41] studied the efficacy of TF as immunotherapy to treat experimental glioblastoma (brain cancer involving glial cells) in rats. TF was obtained from immunized swine and administered at a dose of 4×10^6, 8×10^5 and 1.6×10^5 cells, respectively. The best dose was 4×10^6 cells. TF was also combined with carmustine for experimental therapy in rats with C6 malignant glioma. The authors observed that treating rats reduces the size of the tumor and increases the CD2+, CD4+, CD8+ and natural killer cell counts. The percentage of apoptotic tumor cells and the percentage of tumor tissue expressing Th1 cytokines were also increased. The study demonstrated the beneficial effects of using both TF and chemotherapy, this application having a synergic effect. Therefore, it is possible to decrease the doses of chemotherapy while preserving the same effect of the treatment.

In vitro research has revealed that the ability of lymphocytes to kill cancer cells is aided by transfer factor. DNA fragmentation in MCF-7 breast cancer cells can be caused by DLE obtained from cattle. These DLE can also induce the cytotoxic effect and suppression of some proteins that are associated with apoptosis (TP53, Bag-1, c-Myc, Bax, Bcl-2 and Bad) at the level of mRNA expression in MCF-7 breast cancer cells [42]. Bim mRNA expression was not detected. The extract did not affect the viability of normal mononuclear cells. The extract had dose-dependent cytotoxic effects and demonstrated an IC50 at a dosage of 0.06 U/mL. It induced DNA fragmentation in cancer cells at doses of 0.06 and 0.13 U/mL.

Treatments against cancer with TF were performed about 40 years ago. Fudenberg [43] found that treatment of patients with osteosarcoma with transfer factor derived from selected donors increased cell-mediated cytotoxicity. It appears that treatment with TF can provide prophylaxis against metastases when administered to patients without clinically apparent metastases at the time of surgical removal of the primary tumor. However, initial results from the viewpoint of whether immunotherapy with TF is more effective than chemotherapy were unproven and controversial.

When testing a TF preparation (Transferón®) as an adjuvant to chemotherapy in patients with osteosarcoma in stages III and IV, Juarez [44] recorded an increase in the number of CD3+ CD8+, CD16+ and CD56+ in the blood of the patients. The author also observed that the patients that were treated with Transferón® remained at the same stage without any new metastatic lesions.

Pizza et al. [45] conducted a follow-up investigation, ranging from 1 to 9 years. They were treated with TF 50 patients with prostate cancer unresponsive to conventional therapy. In 44% of them, a beneficial effect was observed (higher survival rates). The investigation showed that complete remission was achieved in 2 patients, partial remission in 5.1, and no progression of metastatic disease in 14. The median survival was 126 weeks, higher than the survival rates reported in the literature for patients of the same stage.

Immunotherapy with TF has been tested in lung cancer patients also. The rationale for using TF in this type of cancer is that the possibility of improving their cell-mediated immunity to tumor associated antigens may improve their survival. Pilotti et al. [46] obtained beneficial results regarding the treatment of lung cancer with TF used as an adjuvant. TF was extracted from the lymphocytes of blood bank donors. During 11 years, 99 non–small cell lung cancer (NSCLC) resected patients were monthly treated with TF. In the same period, 257 NSCLC-resected patients were considered as non-treated controls. The survival rates of the treated patients were significantly improved compared to untreated both for patients in stages 3a and 3b and patients with histological subtype large cell carcinoma. Survival of treated patients is also significantly higher for patients with lymph node involvement (N2 disease). These results suggest that the administration of TF to NSCLC resected patients may improve their survival.

Continuing their previous research, Franco-Molina et al. [47] showed that adjuvant immunotherapy with bovine dialyzable leukocyte extract (in the form of the preparation IMMUNEPOTENT CRP) against lung cancer can cause an immunomodulatory effect. Twenty-four NSCLC patients were included in the study and divided into two groups. Group 1 received a conventional treatment of 5400 cGy external radiotherapy in 28 fractions and chemotherapy consisting of intravenous cisplatin 40 mg/m^2 delivered weekly for 6 weeks. Group 2 received the conventional treatment plus IMMUNEPOTENT CRP (5U) administered daily. The administration of IMMUNEPOTENT CRP induced immunomodulatory activity—increasing the total leukocytes and T-lymphocyte subpopulations CD4+, CD8+, CD16+ and CD56+ and maintaining DHT) and increased the quality of the patients' lives, suggesting immunologic protection against chemotherapeutic side effects in NSCLC patients. These results suggest the possibility of using IMMUNEPOTENT CRP alongside radiation and chemotherapy for maintaining the immune system and increasing the quality of life of the patients.

Therefore, it can be concluded that the TF-preparations, alone or in combination with conventional anticancer treatments can be applied successfully against some types of cancer. However, the scope of research on immunotherapeutic effect of the TF should expand to include other cancers.

5.2. Human infectious, parasitic and allergic diseases

5.2.1. Viral infections

5.2.1.1. Retroviral infections (AIDS and simian AIDS)

Since CMI plays a major role in the control of AIDS, it is considered that TF preparations can be favorable for patients with this disease. DLE can reduce the transcription of HIV-1 and inactivate the NF-κB signaling pathway [32].

The first clinical trial on a TF preparation for AIDS treatment was conducted by Viza et al. [40]. Transfer factor was prepared by immunization of mice with leukocytes from an AIDS patient and replicated in the LDV/7 cell line. In the study, three patients took TF orally for 3–5 months. The results of the treatment were overall clinical improvement and restoration of the skin test reactivity of the patients and a slight increase in their CD4+ cell counts.

Similar results were obtained in the treatment of AIDS patients with non-HIV-specific TF preparations where DTH was restored [48–50] (as mentioned in Ref. [29]). This is associated with the expression of IL-2 receptors of T-lymphocytes [49].

In later research, Pizza et al. treated 25 seropositive patients with mouse-derived HIV-specific transfer factor, which was taken orally by the patients for different time periods ranging from 60 to 1870 days. The results of the treatment were generally favorable; DTH was restored to recall antigen and CD4+ cell counts in 11 of the patients and CD8+ cell counts in 15 of the patients was increased [51]. Such an increase in CD8+ (as well as an increase in the total leukocyte number and the Il-2 level) in AIDS patients after treatment with HIV-specific TF has also been reported by other authors [52].

The cited data indicate that further studies on immunotherapy of HIV infection should be focused on stimulating of cellular, rather than the humoral immune response. This is because despite of the higher expenses for research, the initial hopes and expectations of obtaining an effective anti-HIV vaccine have been dashed for more than 30 years [29].

Both HIV-specific and non-HIV-specific TF preparations can be used as an adjunctive treatment for AIDS. Since the resistance to HIV infection depends on the functional condition of the T cytotoxic lymphocytes, transfer factor can make them "instructed" to resist more effectively. 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 CD8+ T cells is crucial in this process [29].

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 experimental infections with SIV on macaques model. SIV-specific TF was obtained by Viza et al. [53] 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. During a 108-day observation period, the authors found that several hematological and immunological parameters of treated macaques were significantly different from those of the nontreated with TF. The CD4/CD8 ratio, as well as the CD4 cells and platelet counts, showed significant variations between the treated and the non-treated macaques. The animals treated with TF derived from cells enriched with extracts of CD8+ T showed best results.

5.2.1.2. Herpesvirus infections

TF-preparations have been tested on infections with the *Herpes simplex virus* (HSV), Cytomeg*alovir*us (CMV), *Varicella zoster*, Hodgkin's lymphoma and Epstein-Barr virus (EBV) in order to prove their efficiency against them.

Khan et al. [54] were the first to treat HSV with specific TF. In 1981, 16 patients suffering from recurrent herpes—HSV-1 (cold sores) and HSV-2 (genital) were injected with TF on a weekly or monthly basis. The results of this treatment were encouraging—eight patients stopped having outbreaks altogether whereas the remaining eight exhibited a significant reduction in the frequency of outbreaks.

It was also found that the treatment with TF has led to an increase in the number of T lymphocytes; while in approximately half of the patients, it had been reduced prior to the treatment.

The effects of TF against *Varicella zoster* infections have been clinically studied in many works [55–57]. In summary, it has been established that TF serves both as protection and therapy against this type of infections. Compared to untreated patients, patients treated with TF show an increase of CD4+ cells, the γ-interferon level and the CD4/CD8 ratio. The main advantage of TF compared to other antiviral agents is that it induces the production of γ-interferon, which is crucial in the treatment of this infection.

In other herpes infections, transfer factor preparations have shown the best effect against the CMV infection. The treatment with TF develops a cell mediated immune response against CME and disappearance of viremia which leads to dramatic clinical improvements in the patients [29].

The treatment with a specific TF preparation may prevent the re-induction of EVB-induced diseases, but it has no clinical effect against Hodgkin's lymphoma [29].

5.2.1.3. Other viral infections

The effect of specific TF preparations has been tested in cases of infections with other viral diseases, such as viral hepatitis and the human papilloma virus. After treatment of patients with hepatitis B, the biopsy results were encouraging and at the same time, the data were supported by a number of biochemical and immunological indices [58, 59].

Another application of specific TF preparations is their use as an alternative to vaccines against newly occurred deadly influenza viruses. This is due to the fact that they can be produced considerably quickly and because they do not carry the risk of accidents during the production of recombinant vaccine strains of influenza viruses in laboratories [29].

5.2.1.4. Mycobacterial infection

These diseases result from the defect or absence of a cell-mediated immune response to mycobacteria. Thus, it can be presumed that TF could be beneficial in cases of these infections, enhancing the CMI.

Patients who did not respond to conventional therapy were used to trial the effects of TF against *Mycobacterium tuberculosis*. The test was conducted over 40 years ago and the results of the treatment included enhanced CMI reactivity and overall improvement of the participants' clinical condition [60]. Later on, Viza et al. [29] proved that the therapeutic effect of TF against *M. tuberculosis* is dose-dependent. Moreover, TF can be used as adjuvant in cases of ganglionic and cutaneous tuberculosis resistant to conventional treatments [61].

The mechanisms of action of TF against *M. tuberculosis* were studied using the mouse model. The specific TF was produced from tuberculous BALB/c mice following intra-tracheal infection. The treatment with TF leads to restoration of the expression of the Th1 cytokine pattern, whereas the increase of delayed type hypersensitivity leads to inhibition of bacterial proliferation and animal survival [62].

TF preparations against *Mycobacterium leprae*, *Mycobacterium fortuitum pneumonia* and *Mycobacterium xenori* were also tested (quote by Viza et al.) [29]. Treatments with TF preparations to patients suffering from leprosy were performed for a fairly long time (over 40 years). Improvement in the condition of patients was achieved, but the results were not convincing.

5.2.1.5. Fungal infection

TF preparations were obtained and tested against chronic mucocutaneous candidiasis, coccidiodomycosis and fungal keratitis.

The treatment showed a positive effect on *Candida albicans*, increasing the immunological reactivity of the treated patients [63].

Graybill et al. [64] treated three patients with progressive coccidioidomycosis. Prolonged clinical remission was found in two of them. Comparable results have been obtained by Catanzaro et al. [65].

Thirty-three-year-old man with fungal keratitis was treated with dialyzable leukocyte extract as adjuvant. After TF therapy, it was observed diminished infiltration of the corneal stroma and epithelial healing; interestingly, the systemic Immunological changes characterized by increased frequency of IFN-g+ cells were coincident with the clinical progress observed in the patient [66].

5.2.1.6. Parasitoses and allergies

TF have been used in cutaneous leishmaniosis, cryptosporidiosis (in AIDS patients) and echinococcosis [29, 67–70].

Several months of therapy against leishmaniosis with TF has led to a considerable healing of the lesions in patients whose disease had persisted for 8–30 years [67].

When treated with TF against cryptosporidiosis, a decrease in bowel movement frequency was found, as well as a significant weight gain, with eradication of oocytes from the stool in two of them [70].

Influencing these diseases is associated with increasing the CMI response [68], inducing the formation of IFN-γ and inhibiting of IL-5 synthesis [69].

Transfer factor may be a beneficial adjuvant in the treatment of allergic rhinitis [71] and atopic dermatitis [72].

TF-preparation (Transferon) induced some low-frequency non-serious adverse events during adjuvant treatment of patients with immune-mediated diseases [73].

5.3. Animal infectious and parasitic diseases

The large economic losses caused by outbreaks in domestic animals necessitated the search for new approaches to reduce and control them. That is why almost immediately after the first attempts to use transfer factor in human medicine, experiments with TF were also initiated in veterinary medicine [2, 7]. The first experiments carried out in this area were with TF against parasites and intracellular microorganisms.

The first publications on testing TF preparations in the field of animal health are for treating coccidiosis. Liburg et al. [74] have shown that treatment with specific DLE from rats, which have been experimentally infected with *Eimeria nieschulzi*, causes a reduction in the number of oocysts in their feces. Similar results were received for other types of coccidia in domestic animals by Klesius and Kristensen [75] and Klesius and Giamborne [76]—the oocysts in the feces were reduced. Specific TF preparations were used to treat *Eimeria bovis* in calves and *Eimeria tenella* in chickens.

The specific DLE preparations can be successfully used in ruminants to prevent nematodoses. Furthermore, *Trichostrongylus axei, Trichostrongylus colubriformis, Ostertagia circumcinta, Ostertagia ostertagi* and *Haemonchus controtus* were the focus of research on the action of both nondialyzed leucocytes lysate and dialyzable TF preparations. The worm burden was significantly reduced as a result of the treatment with DLE. Furthermore, nondialyzed leucocytes lysates proved to be more effective compared to dialyzable transfer factor preparations [7].

Dialyzable leukocyte extracts were also tested for their ability to prevent salmonella infections in domestic animals. The first successful studies on the protective effect of TF against *Salmonella typhimurium* were performed on mouse model [17, 77]. Later, Mikula et al. [78] and Mikula et al. [2] reported bacteriological, immunological and clinical trials of specific and non-specific DLE on calves which have been experimentally infected with a virulent strain *Salmonella typhimurium*. Injecting the calves intravenously with specific DLE protected them against the experimental infection with a pathogenic strain *S. typhimurium* 4/5. The number of salmonellas in the feces of the calves was reduced, the phagocytic activity of the leukocytes was activated, the number of lymphocytes in the peripheral blood was increased and specific CMI was developed—which proves the protective action of the specific DLE.

The activity of specific preparations against experimental infection with *Salmonella choleraesuis* was tested using the mouse model [25]. The treating of the mice with DLE induced a high specific protective effect (70%). Diffuse proliferation of activated macrophages in the lamina propria of the small intestine at the place of penetration of Salmonellas has been observed. The proliferation of activated macrophages is a manifestation of cell-mediated immune response to Salmonellas in the penetrated tissues [79].

DLE-preparation has also been tested against natural Salmonella infection in pigs by us. Treatment of animals has led to limiting morbidity and coping with the infection at the farm. The effectiveness of specific DLE against infections with *Salmonella enterica* subsp. *enteritidis*

in chickens has also been examined [80]. It has been found that the treatment of chickens with DLE reduces the presence of *S. enterica* in the caecum.

The protective effect of the transfer factor preparation against some viral and bacterial infections in piglets was examined [81]. The preparation was obtained from the lymph nodes of pigs immunized against the causative agents of respiratory and gastrointestinal diseases as piglets. Treatment with the preparation displayed a very good protective effect, triggering a significant reduction in morbidity and mortality in the treated piglets.

Similar results were obtained recently in Mexico, where a research group has received highly purified swine dialyzable spleen extract with antiviral activity tested on mouse model [82].

When applied to pigs, DLE considerably increased the IFN-γ concentration in their serum as measured 30 days after the treatment. This can be used to limit the cases of diarrhea and respiratory diseases among the animals—which would lead to definite improvements since these are both problematic diseases in weaned pigs. The application of porcine DLE has a good effect on weaned pigs [31].

It was received and tested TF preparation against avian influenza [83]. It has been proven that the treatment of chickens with specific TF, alone or combined with a vaccine against avian influenza, induces the expression of IFN-γ and IL-2.

6. Dosage of TF preparations

One unit of TF preparation is equal to the product obtained from 5×10^8 leukocytes. The dosage of TF in immunotherapy depends on the patient's characteristics, the disease and the therapeutic response.

Berrón-Pérez et al. [9] offer a few basic schemes for immunotherapy with TF—for initial treatment for acute and chronic diseases and for short- and long-term maintenance treatment. The authors offer various schemes in infectious, non-infectious diseases (allergic and autoimmune) and special diseases (sepsis, major surgery and cancer).

7. Conclusion

It can be summarized that dialyzable leukocyte extracts containing transfer factor are promising immunotherapeutic agents. In a large number of experiments, their protective and therapeutic effect has been proven against a number of diseases in humans and domestic animals. Understanding the mechanism of action of DLE and determining appropriate dosages determine the positive effects of their application against a very wide range of diseases—neoplasm, immune deficiencies, infectious, parasitic and autoimmune diseases.

The low level of difficulty of the process of obtaining protocols determines their relatively low cost and the possibility to combine them with other therapeutic agents during treatment makes them subject to medical applications in the future, including against some new diseases.

Acknowledgements

I dedicate this paper to people with whom I have worked - Igo Schröder (Rostock, Germany), Josef Crejci (Brno, Czech Republik), Juraj Pistl (Kosice, Slovak Republic), Georgi Ignatov (Plovdiv, Bulgaria), Stefan Boyadzhiev (Plovdiv, Bulgaria) and Nicola Tsiporkov (Plovdiv, Bulgaria).

This chapter is published with the support of NPD – Plovdiv University "Paisii Hilendarski" underProject №: PP-2016-3.

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