Dialysable leukocyte extracts in immunotherapy

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Dialysable leukocyte extracts (DLE) are complexes consisting of a large number of low-molecular-weight substances. These extracts have immunomodulatory properties, which are mainly attributed to small peptides commonly referred to as ‘transfer factor’. This review focuses on the characteristics of DLE with transfer factor activity, together with the methods for their preparation and purification. The opportunities for applying the extracts for immunotherapy purposes against various diseases in humans and domestic animals are also discussed. Their ease of preparation and relatively low cost combined with the rapid positive effect they produce will make DLE subject to application in medicine in the future, including their use against new nosological entities.

Keywords: cytokines; dialysable leukocyte extract; diseases; immunotherapy; transfer factor

Abbreviations

- CD: cluster of differentiation
- CMI: cell-mediated immunity
- DLE: dialysable leukocyte extracts
- DNase: deoxyribonuclease
- DTH: delayed-type hypersensitivity
- HBV: hepatitis B virus
- hBD-2: human beta-defensin-2
- HIV: human immunodeficiency virus
- OD: optical density
- LL-37: cathelicidin
- MCF-7: Michigan Cancer Foundation-7
- mRNA: messenger ribonucleic acid
- RNase: ribonuclease
- TF: transfer factor
- Th: T helper cells

Introduction

The discovery that it is possible to transfer cell-mediated immunity (CMI) to naïve recipients by leukocyte derivatives gave a new opportunity to medicine. Since CMI is crucial for controlling infections, as well as cancer, autoimmune diseases, immunodeficiencies and allergies, TF can be used in the prophylaxis and treatment of these diseases.[1,3,5–8]

This review focuses on the nature and immunological characteristics of DLE containing TF, their mechanism of action and the possibilities for them to be used as immunomodulators in human and veterinary medicine.

Composition, physicochemical and biochemical properties of DLE

Transfer factor, the main component of the DLE, is composed of small peptides with a molecular weight of 3.5–6.0 kDa, to which oligoribonucleotides are attached.[3] The specific activity of TF is due to peptides of 5.0 kDa.[9] In 2000, while analysing the peptide partial sequences of TF, Kirkpatrick [10] found a novel amino acid consensus sequence LLYAQDL/VEDN, which 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.[7] The N-terminal region of these peptides is very similar to some neuropeptides, such as the enkephalins.[11]

Apart from TF, the extracts also contain cyclic nucleotides, ascorbate, prostaglandins, histamine, serotonin, nicotinamide and some amino acids and purine bases.[12] DLE preparations are transparent, pyrogen-free, light...
Mechanism of action and immunological properties of transfer factor

TF is produced by CD4⁺ Th1 cells during the immune response to an antigen.[8,18] Its biological activity includes different – sometimes opposite – effects. Transfer factor contains the following constituents: antigen-specific inducer, suppressor [8] and non-specific (adjuvant-like) components [19]. In the extracts, there can also be detected fractions with hematopoietic activity,[20] as well as in vitro antibacterial activity.[21]

The antigen-specific components are informational molecules involved in the immune recognition of antigens that have entered the body and in the formation of immune memory. The inducer fractions enhance the antigenic stimulus, which causes the production of interferon gamma (IFN-γ), interleukin (II) 2 (II-2) and tumour necrosis factor alpha (TNF-α) by CD4⁺ Th1 cells. As a consequence, cell-mediated immune response develops against the target antigen.[8] and it includes interleukins (II-6 and II-8) formed by activated monocytes.[22,23] According to Ojeda et al. [22], the regulation of the production of TNF-α, II-6 and II-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. Subsequently, however, the role of the ligands for TLR4 as regulators of the production of these cytokines was excluded.[24,25]

Suppressor fractions take part in the regulation and attenuation of the immune response to an antigen by stimulating the formation of IL-10 and inhibitory cytokines by Th2 cells.[8,18,26] The adjuvant-like components of TF have a non-specific activity expressed by enhancing the immune response to other antigens or allergens.[19] Thus, TF takes 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.

Transfer factor could be ‘replicated’ in the lymphocytes of a naïve recipient. The lymphocytes of the recipient act as an efficient copier by integrating the specifics of the injected TF, and thereby effectively converting the recipient into a TF donor. This allows to obtain TF-preparations from donors infected with an unknown pathogen, a phenomenon referred to as ‘the black box effect’ (for review see Viza et al. [27]).

Another mechanism of action of DLE is via regulating 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 critical role in the innate immune defense against invasive bacterial infection and inflammation.

Obtaining transfer factor preparations

The first TF preparations were obtained in the laboratory of Lawrence though dialysis of human leukocyte cryosates.[4] Furthermore, dialysis as a separation method of low-molecular-weight components was replaced by ultrafiltration.[17,20,29] Membrane filters with a cutoff of <12 kDa are used in the procedure. Apart from human leucocytes, TF preparations are obtained from ultrafiltrated animal cryoysed leucocytes or lymphoid organs (lymph nodes and spleen).[16,17,20,30,31]

Transfer factor can also be produced in bird eggs. Xu et al. [32] have obtained TF from egg yolk which is active against HBV and Hennen and Lisobree [33] have patented a method for producing TF preparations from bird eggs.

Another source of TF is bovine colostrum. The method was developed by Wilson et al. [34] and currently has very wide application, since 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 derived from bovine colostrum is patented as a medicament.[35]

DLE may be further subjected to purification using column chromatography, high-performance liquid chromatography [8,36] and molecular exclusion liquid chromatography [37]. Through these procedures not only a better purification of the preparations can be achieved, but also different fractions can be isolated.

Using DLE preparations in human and veterinary medicine

The application of preparations containing TF in medicine is based on the influence of TF on the function of various immune components and also on the regulation of cytokine synthesis. Each pathogen can cause the formation of yellow fluids, with pH 5.5–7.0 or lower (pH 5.6–6.8) depending on the method of obtaining.[2,8,13,14] The ratio OD260/OD280 (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.[14–17] The index values show that nucleotides are relatively predominant over peptides. The osmolarity of DLE preparation was measured by Grob et al. [14] and has a value of 520 mOsm/L. TF is resistant to treatment with DNase, pancreatic RNase and trypsin, but cannot withstand snake venom phosphodiesterase.[8] TF can resist deep freezing, but is heat labile. Its heat sensitivity depends on the melting of the double-stranded nucleic acid. The biological activity of DLE preparations is retained for several years when stored at a temperature between −20 °C and −70 °C and is lost at 90 °C.[8] As it is a low-molecular weight mixture, DLE is not immunogenic and contains no histocompatibility antigens.[3,8]
TF; at least one TF is created for each piece of pathogen that the immune system interacts with.[38]

An important advantage of TF preparations as therapeutic agents is that they induce a rapid immune response against the pathogen (within 24 hours) and thereby reduce the time for the patient immune response by 9–13 days (the time needed for complete cell-mediated immune response against a pathogen is 10–14 days).

Another aspect of TF application in medicine is based on the fact that major diseases, such as cancer, rheumatoid arthritis, hepatitis, heart diseases, Alzheimer’s etc., are caused by abnormalities in the formation of the TF of CD4+Th1 cells. Continuing their previous research, Franco-Molina et al.[45] showed that adjuvant immunotherapy with bovine dialysable leukocyte extract (in the form of the preparation IMMUNEPOTENT CRP) against lung cancer can cause an immunomodulatory effect (increasing the total leukocytes and T-lymphocyte subpopulations CD4+, CD8+, CD16+ and CD56+), maintaining DTH and increase the quality of life of the patients.

**Cancer**

There are about 100 reports on the effect of TF on cancer. Pineda et al. [39] observed that treating rats that have C6 malignant glioma with swine TF preparation significantly reduces the tumour size and increases the CD2+, CD4+, CD8+ and natural killer cell counts. It also increases the percentage of apoptotic tumour cells and the percentage of tumour tissue expressing Th1 cytokines. This study showed the benefits of combining TF with chemotherapy because of the synergic effect. Thus, the chemotherapy doses can be decreased and at the same time the effect of the treatment can be maintained.

*In vitro* research has shown that TF facilitates the ability of lymphocytes to kill cancer cells. Bovine DLE can cause DNA fragmentation in MCF-7 breast cancer cells and can induce the cytotoxic effect and suppression of some proteins that influence apoptosis (TP53, Bag-1, c-Myc, Bax, Bcl-2 and Bad) at the level of mRNA expression in MCF-7 breast cancer cells.[40] The extract did not affect the viability of normal mononuclear cells.

The first experimental clinical treatments against cancer with TF were performed about 40 years ago. Fudenberg [41] found that treatment of patients with osteosarcoma with dialysable TF increased cell-mediated cytotoxicity.

When testing a TF preparation (Transferon®) as an adjuvant to chemotherapy in patients with osteosarcoma in stages III and IV, Juarez [42] observed an increase in the number of CD3+ CD8+, CD16+ and CD56+ in the blood of the treated patients. It is also reported that the patients treated with Transferon® remain at the same stage and no new metastatic lesions are observed.

Pizza et al. [43] treated with TF 50 patients with prostate cancer unresponsive to conventional therapy. In 44% of them, a beneficial effect was observed (higher survival rates). Pilotti et al. [44] also obtained similar results regarding the treatment of lung cancer with TF used as an adjuvant. The authors report that the survival time of the patients treated with TF was longer than that of the ones who had not been treated.

Human infectious, parasitic and allergic diseases

**AIDS**

Since CMI plays a major role in the control of AIDS, it is considered that TF preparations can be favourable for patients with this disease. DLE can reduce the transcription of HIV-1 and inactivate the NF-κB signalling pathway.[31]

The first clinical trial on a TF preparation for AIDS treatment was conducted by Viza et al. [46]. Murine HIV-specific TF was orally administered to three patients for a period of 3–5 months. The treatment resulted in clinical improvement of the patients as well as in restoration of their skin test reactivity and a moderate increase in their CD4+ cell counts.

Similar results (restoration of DTH) were obtained in the treatment of AIDS-patients with non-HIV-specific TF preparations [47–49] (as mentioned in [27]). The restoration of DTH is associated with expression of IL-2 receptors of T-lymphocytes.[48]

Later, Pizza et al. [50] performed oral treatment of 25 seropositive patients with mouse-derived HIV-specific TF for a period of 60–1870 days. There was a beneficial effect in most treated patients, accompanied by restoration of DTH to recall antigen and an increase in CD4+ cell counts in 11 patients and CD8+ cell counts in 15 patients. [50] Such an increase in CD8+ (as well as an increase in the total leucocyte number and the II-2 level) in AIDS patients after treatment with HIV-specific TF has also been reported by other authors.[51]

Based on the above, it can be concluded that both HIV-specific and non-HIV-specific TF preparations can be used as an adjunctive treatment for AIDS. Application of TF preparations leads to recovery of CMI (the HIV target).

**Herpesvirus infections**

There have been tests on whether TF preparations are efficient against infections caused by Herpes simplex virus (HSV), Varicella zoster, Cytomegalovirus (CMV), Epstein–Barr virus and Hodgkin’s lymphoma.

The first treatment with specific TF against HSV was performed by Khan et al. [52] in 1981 on 17 patients suffering from recurrent herpes. The preliminary results of this treatment were encouraging, which is why later other
researchers have successfully performed similar treatments against HSV (as mentioned in [27]).

Steele et al. [53], Bowden et al. [54] and Estrada-Parra et al. [55] carried out clinical trials on specific TF against Varicella zoster infections. It has been found that the TF has both protective and therapeutic effect against this infection. This is associated with the increase in CD4+ cells, the γ-IFN level and the CD4/CD8 ratio in patients treated with TF compared to untreated patients.

In other herpes infections, TF preparations have shown the best effect against the CMV infection. Treatment with TF develops a cell-mediated immune response against CMV which leads to dramatic clinical improvements in the patients.[27]

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.[27]

Other viral infections
TF preparations have been tested against other viral diseases, such as viral hepatitis and human papilloma virus infections. Patients with hepatitis B were treated with a specific TF preparation and the results of their biopsy examination, as well as of a number of biochemical and immunological indices, showed favourable results.[56,57]

What is more, according to Viza et al. [27], specific TF preparations could be used as an alternative to vaccination against new deadly influenza viruses because TF can be produced very quickly and because there is no risk of accidents during the laboratory production of recombinant vaccine strains of influenza viruses.

Mycobacterial infection
Mycobacterial disease occurs in the absence or defect of the cell-mediated immune response to mycobacteria. It is therefore expected that TF could have a positive effect in these infections, enhancing the CMI.

The first test of TF against Mycobacterium tuberculosis was conducted over 40 years ago on patients who do not respond to the conventional therapy. Treatment with TF led to the development of CMI reactivity and improvement of their clinical condition.[58] Later on, Viza et al. [27] proved that the therapeutic effect of TF against M. tuberculosis is dose-dependent. Moreover, TF can be used as adjuvant in cases of ganglionar and cutaneous tuberculosis resistant to conventional treatments.[59]

The mechanisms of action of TF against M. tuberculosis have been studied using a mouse model. They are related to restoration of the expression of the Th1 cytokine pattern, increase in DTH, leading to inhibition of bacterial proliferation and better animal survival.[60] TF preparations have also been tested against M. leprae, M. fortuitum pneumonia and M. xenori (as mentioned in [27]).

Fungal, parasitic and allergic diseases
TF preparations have been obtained and tested against Candida albicans,[61] coccidiodomycosis [62,63] and fungal keratitis [64]. The treatment showed a beneficial effect.

TF have been used in cutaneous leishmaniasis, cryptosporidiosis (in AIDS patients) and echinococcosis.[27] Influencing these parasitoses is associated with increasing the CMI response,[65] induction of IFN-γ formation and inhibition of IL-5 synthesis [66].

According to Homberg et al. [67], DLE may be a beneficial adjuvant in the treatment of allergic rhinitis. Similar results were obtained by García et al. [68] when testing DLE as an immunomodulator treatment in atopic dermatitis.

The possible adverse events in patients treated with DLE (transferon) have been studied by Homberg et al. [69]. Transferon induced some low-frequency non-serious adverse events during adjuvant treatment of patients with immune-mediated diseases.

Using DLE preparations in veterinary medicine
The major economic losses caused by mass infectious and parasitic diseases in domestic animals have served as a driving force in the search for new approaches to their reduction and control. That is why almost immediately after the first attempt to use DLE in human medicine (in the 1970s), experiments were also initiated with DLE with a view to protecting and treating domestic animals. [2,5] The theoretical basis lays in the ability of TF to activate the CMI, which directed the experimenters to test certain preparations against protozoa, helminthes and intracellular pathogens.

The first reports on testing TF preparations in the field of veterinary medicine are for treating coccidiosis. Liburd et al. [70] showed that, in rats experimentally infected with Eimeria nieschulzi, treatment with specific DLE causes a reduction in the number of oocysts in the faeces. Later, Klesius and Kristensen [71] and Klesius and Giamborne [72] reported similar results obtained for other types of coccidia in domestic animals (a reduction of oocysts in the faeces), using specific TF-preparations for the treatment of E. bovis in calves and E. tenella in chickens, respectively.

DLE preparations can be applied with success in preventing nematodoses among ruminants. The action of both non-dialysed leucocyte lysate and dialysable TF preparations has been tested against Trichostrongylus axei, Trichostrongylus colubriformis, Ostertagia circumcinta, Ostertagia ostertagi and Haemonchus contortus. The treatment with DLE highly reduced the worm burden, and non-dialysed leucocytes lysates were more effective than dialysable TF preparations (as mentioned in [5]).
DLE have also been tested for their ability to prevent Salmonella infections in domestic animals. In 1982, Smith et al. [73] performed the first successful studies on the protective effect of the murine TF against Salmonella typhimurium using the mouse model. Later, Mikula et al. [74] and Mikula et al.[2] reported bacteriological, immunological and clinical trials of specific and non-specific DLE on calves experimentally infected with a virulent strain S. typhimurium 4/5. Injecting the calves intravenously with specific DLE protected them against the experimental infection. The protective action of the specific preparation is expressed by a reduction in the number of Salmonellas in the faeces of the calves, activation of the phagocytic activity of leucocytes, an increase in the number of peripheral-blood lymphocytes and development of specific CMI.

The protective activity of rabbit DLE against Salmonella choleraesuis has been examined using the mouse model.[75] Treating mice with DLE induced specific protection. Diffuse proliferation of activated macrophages in the lamina propria of the small intestine at the place of penetration of Salmonellas has been observed in experimental infection with a pathogenic strain.[76] The proliferation of activated macrophages is a manifestation of cell-mediated immune response to Salmonellas in the penetrated tissues.

Kokincakova et al. [77] examined the protective activity of specific DLE against infections with Salmonella enterica subsp. enteritidis in chickens. The authors report that treatment of chickens with DLE reduces the presence of S. enterica in the caecum.

Hernandez-Peralta et al. [30] report that the application of porcine DLE has a good effect on weaned pigs. Intradermal application of DLE in pigs significantly increased the IFN-γ concentration in the serum 30 days after the treatment. This effect is a prerequisite for reducing the cases of diarrhoea and respiratory diseases among treated animals — both problematic diseases in weaned pigs.

The possibility of using a TF preparation against avian influenza has been examined.[78] It has been shown 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.

Testing TF preparations in the field of veterinary medicine has given promising results, which suggests that they will be used more widely in future.

Conclusions

Based on the data discussed above, it can be concluded that DLE containing TF are a reliable immunomodulator which has a protective and therapeutic effect against a number of diseases. Insight into the mechanisms of action of these extracts will determine the correct approach in their application against cancer, immunodeficiency, autoimmune, infectious and parasitic diseases. Their ease of preparation and relatively low cost combined with the rapid positive effect they produce are likely to make them subject to application in human and veterinary medicine in the future, including their use against nosological entities that have not been treated with DLE.

Disclosure statement

No potential conflict of interest was reported by the authors.

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