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An overview on immunological activity of calf thymus extract TFX® and its therapeutic benefits

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Abstract: This review summarizes available literature regarding immunoregulatory activities of calf thymus factor X (TFX®) in vitro and in vivo, both in animal and human models, as well as its therapeutic efficacy evaluated in a number of clinical trials. TFX® is a safe, non-toxic preparation of thymic hormones from juvenile calves' thymic glands, capable of restoring immune system functions in immunocompromised animals and patients by acting not only on immature T cells but also indirectly on maturation and function of other cell types. A potential, preventive or therapeutic utility of TFX® could find most optimal application in combating immunodeficiency, autoimmune disorders and restoration of full immune competence following chemo- or radiotherapy. More research is needed to explain its molecular and cellular mode of action after a long period of inactivity in this field.

Keywords: TFX®, calf thymus extract, immunotherapy, respiratory tract infections, immunosuppression, viral infections, autoimmune diseases, anticancer therapy

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1. Introduction

Search for new, safe therapeutics creates an enormous challenge for medicinal chemistry and pharmacology. Of a particular demand is treatment of inflammatory diseases, cancer, autoimmune responses, immunodeficiency and restitution of the immune system function following chemotherapy. Although the problem could be partially solved by application of synthetic compounds [Rejas et al., 1988; Zimecki et al., 2012; Zimecki et al., 2015], growth factors [Barrientos et al., 2008] or recombinant cytokines [Dale et al., 2018], as early as in mid 70-ties, extracts or more defined fractions from thymuses were proposed as potentially useful therapeutics for treatment of such diseases or clinical states [Basch & Goldstein, 1975; Kook et al., 1975]. Thymic factors, together with cytokines and growth factors are collectively termed as biological response modifiers (BRMs) [Goldstein & Goldstein, 2009]. They are capable to affect impaired or deregulated immune response by reconstitution of particular types of immune cell deficits and enhancement or correction of the immune response. Thus, application of BRMs is of particular importance in therapy of diseases where malfunction of the immune system generates pathological processes. BRMs are applied alone or in combination with conventional therapy to enhance its efficacy or to weaken side effects of such treatment.

Among the thymic hormones, thymosin α1 and thymosin β4 were identified by Allan L. Goldstein [Goldstein et al., 1972], thymopoietin by Gideon Goldstein [Goldstein et al., 1979] and serum thymic factor by Jean-Francois Bach [Bach et al., 1975]. Until now, thymic hormones and their synthetic versions have been

in use to treat inflammatory diseases and sepsis [Lunin & Novoselova, 2010; Pei et al., 2018], multiple sclerosis [Severa et al., 2019], HIV infection [Matteucci et al., 2017], viral hepatitis [Chan et al., 2001] or cancer [Constantini et al., 2019; Lunin & Novoselova, 2010; Skotnicki, 2010; Skotnicki, 2019].

Also, in Poland attempts have been undertaken to isolate from calf thymuses a therapeutically applicable preparation – Thymus Factor X (TFX Thymostimulinum®), a partially purified aqueous extract of juvenile calves' thymic glands (constituting a nucleotide- and lipid-free fraction of polypeptides of MW below 10 kDa, with a major component of 4.2 kDa) [Czarnecki & Jaskólski, 1978]. Among peptides included in TFX® preparation thymosin α1 and thymosin β4 were also found [Skotnicki, 1989b]. TFX® specimen was worked out in 1976, patented in 1981 (main researcher Jan Czarnecki) and manufactured initially (since 1983) by Polfa Pharmaceuticals, Jelenia Góra, Poland, later by Finepharm Ltd., Jelenia Góra, and was subjected to a number of studies, both in animal and human models. In many cases TFX® was used in patients refractory to classical treatment or in combination with generally approved therapeutics. In the last decade experiments in animal models and for two decades clinical studies on patients on TFX® virtually ceased. In the latter case new, strict ethical regulations imposed by the European Union, related to clinical trials, were crucial. At present Thymus Factor® – freeze-dried peptides from calves' thymic glands enriched with vitamin C, zinc and selenium manufactured by TFX Pharma, Wroclaw, Poland is available as diet supplement.

The aim of this article is to overview hitherto available literature regarding research on calf thymus extract as TFX® specimen, both in animal and human models, and to propose new therapeutic perspectives for the preparation. Since the therapeutic benefits of TFX® application in clinical trials, conducted by year 1988, have been already presented in detail in a review article [Skotnicki, 1989a], they will be referred to shortly in this review. Hence, for more detailed description of experimental protocols, results and references regarding these clinical trials, we encouraged the readers to see the above-mentioned review article. More information about TFX® application in patients with disorders of the immune system after conventional antineoplastic therapy may be also found in another review article [Skotnicki, 2010].

It is worth to mention that similar studies obtained by Polish investigators were performed by researches in other European countries, USA, Israel, Japan and China, with analogous thymic preparations (peptides isolated from calf thymuses or synthetic versions of thymus-derived peptides), assigned as: Thymosin α1 (Tα1), Thymomodulin (TMD), Thymostimulin (TP-1), Thymopoietin (TP), Thymopentin (TP-5), Thymulin (FTS-Zn), Thymic Humoral Factor (THF) and others [Constantini et al., 2019; Cordero et al., 1997; Cunningham-Rundles et al., 1994; Goldstein & Goldstein, 2009; Lunin & Novoselova, 2010; Pei et al., 2018; Severa et al., 2019; Skotnicki, 2010; Skotnicki, 2019].

2. Current state of knowledge on TFX®

2.1. *In vitro* and *in vivo* studies in animals

In animal models of acute and chronic toxicity (mice, rats, rabbits, hamsters, guinea pigs and cats) TFX® was devoid of toxicity except high intravenous doses, thus recommended for *in vivo* trials in patients. The only exception were women in the reproductive period. As the authors concluded, "TFX® is a preparation acing selectively and exhibiting high specificity to the lymphatic system, and secondarily to the immune system. TFX® may be thus a valuable hormonal drug interfering in the disturbed immune processes" [Ślopek et al., 1980]. High doses of Thymomodulin-TFX®, several times exceeding these proposed for clinical treatment, were used in guinea pigs, rabbit and rats to evaluate its effect on several physiological processes such as pain response to acetic acid injection, hexobarbital sleeping time, rabbit skeletal muscle response and reproduction of albino rats. Thymomodulin-TFX® administration had no undesirable effects on the examined physiological parameters and reproduction processes [Kosmala et al., 1993].

A next series of investigations related to immune reconstituting effects of TFX® in hydrocortisone (HC)-treated and stressed mice. In a first study TFX® was shown to be immunotropic in thymectomized mice and counteracted cytolytic action of HC [Giełdanowski et al., 1980; Giełdanowski & Ślopek, 1981]. In a next study, TFX® was investigated in parallel with sodium diethyldithiocarbamate (DTC, a low molecular weight sulphur compound that may function as a thymic hormone to induce maturation of T-lymphocytes) in the humoral immune response to sheep red blood cells (SRBC) in mice subjected to immobilization stress. TFX® or DTC, administered intraperitoneally (i.p.) either before or after exposure to stress, reversed inhibition of antibody forming cell number and serum antibody titers in stressed mice [Obmińska-Domoradzka & Debowy, 1996]. Furthermore, in a similar model TFX® or DTC, administered i.p. before restrain stress exposure, were shown to counteract the suppressive effects of stress on the capability of thymocytes to proliferate in response to mitogens: concanavalin A (ConA) or phytohemagglutinin (PHA). TFX® had a better reconstituting potency than DTC [Obmińska-Domoradzka, 1997]. In the same mouse model, the i.p. treatment with TFX®

per se, or with zinc ions (added to drinking water), prevented immunosuppressive effects of restrain stress on cell numbers in lymphoid organs, lipopolysaccharide (LPS)-induced interleukin 1 (IL-1) production by peritoneal macrophages and proliferative response of thymocytes stimulated with ConA and PHA. Zinc supplementation augmented effect of TFX® on the cellular response of mice [Obmińska-Mrukowicz & Szczypka, 2005]. In another study administration of HC strongly decreased number of thymocytes, splenocytes, percentage of CD4+, CD8+ and CD19+ splenocytes and CD4+CD8+ thymocytes, as well as IL-1 production by peritoneal macrophages stimulated with LPS. All these changes were reversed by application of TFX® or DTC i.p. prior to HC injection. TFX® also corrected mitogenic responses of thymocytes and ability of peritoneal macrophages to produce IL-1. Simultaneous zinc supplementation in drinking water augmented immunorestorative effect of TFX® [Obmińska-Domoradzka et al., 2002].

Apart of being capable of reconstituting the immune functions in thymectomized mice, TFX® was also protective in restoration of the immune function of guinea pig splenocytes after irradiation [Kowalczyk-Bronisz & Paegelow, 1986]. In a study in dogs and mice the effects of crude TFX® and its immunologically most active V fraction was tested for their hemodynamic and hemopoietic activity. The hemodynamic function of TFX®, but not of fraction V, was observed. On the other hand, the actions on hemopoietic stem cells in mice were observed by application of both preparations [Raberger & Giełdanowski, 1985].

An interesting study regarded effects of thymic extracts on activity of cerebral superoxide dismutase (SOD) and malonyl dialdehyde (MDA) levels in brain of 450-day old mice. The treatment with thymic extracts resulted in a decreased concentration of MDA in brain and, as compensatory effect, decreased activity of SOD. An idea of delaying process of aging by thymic extracts seems to be attractive [Błońska et al., 1989].

A number of articles was devoted to therapeutic effects of TFX® in the model of *Trichinella spiralis* infected mice. In a first investigation, mice infected with *T. spiralis* larvae and subsequently treated subcutaneously (s.c.) with TFX-Thymomodulin® (TFX-Th®, Thymoorgan GmbH Pharmazie Co. KG, Vienenburg, Germany) demonstrated effective removal of larvae from muscles. TFX-Th® was effective if administered at an early, as well as a late stage of *T. spiralis* larvae invasion [Iwanow, 1994]. Next studies regarded mice infected with *T. spiralis* followed by treatment with: TFX®, purified PHA (PHA-P, phytohaemagglutinin from *Phaseolus vulgaris*) or dexamethasone (DEX), and monitored for number of inflammatory cells penetrating through larvae capsules. Better inflammatory infiltrations were observed in PHA-P or TFX®-treated mice than in control (untreated) mice. Fewer cells were found in the DEX-treated mice, that could account for its lack of effectiveness in larvae eradication [Karmańska & Piekarska, 2001]. In a subsequent, similar study with trichinellosis, the authors showed that PHA-P elicited the process of apoptosis in the jejunum mucosa and prolonged it in the muscular inflammatory cell infiltration, TFX® had no effect on this process and DEX even decreased the content of apoptotic cells. The cellular inflammatory infiltrations in the muscles were larger in mice treated with PHA-P or TFX® and smaller in mice treated with DEX, in comparison to control animals. The authors were of opinion that extent of cell infiltration, and not degree of apoptosis of infected cells, played a major role in removal of *T. spiralis* larvae [Karmańska et al., 2001]. The effects of TFX® on reduction of *T. spiralis* larvae and proportions of apoptotic and necrotic lymphocytes in the spleen, mesenteric lymph nodes and muscle of infected mice, were also studied. TFX® administered s.c. repeatedly after infection increased number of apoptotic lymphocytes and decreased parasite larvae load [Piekarska et al., 2009]. The effects of TFX®, LPS and DEX on apoptosis and necrosis of lymphocytes in inflammatory infiltrations in the mouse muscle tissue were analyzed in the next study of the same authors. The best results were obtained with TFX® in terms of changes in proportion of apoptotic and necrotic lymphocytes, as well as removal of *T. spiralis* larvae load. DEX increased the parasite load that could be expected from such a strong immune suppressor [Piekarska et al., 2010]. In a similar study, infection with *T. spiralis* decreased the percentage of CD8+ T cells in the spleens but not in the mesenteric lymph nodes. On the other hand, the percentage of CD4+ cells in the spleens and mesenteric lymph nodes was elevated. Also, in basal lamina propria of the jejunum and inflammatory infiltrates of the muscle tissue, increases of CD4+ and CD8+ cell numbers were found. The i.p. pretreatment with TFX® generally potentiated the phenotypic changes elicited by *T. spiralis* infection in the spleen, mesenteric lymph node, jejunum and inflammatory infiltrates of the muscle [Obmińska-Mrukowicz et al., 2002].

TFX® alone, or in combination with antibiotics, was also tested for its capability to combat bacterial (*Escherichia coli* or *Staphylococcus aureus*) infections in mice. The combined therapy with antibiotics provided various, not conclusive results. However, TFX® administration alone was effective in mice infected with *E. coli*, but weak in *S. aureus* infection [Ciebieda et al., 1992].

A part of the studies was focused at effects of TFX® on reproductive processes in animal models. As mentioned above, Thymomodulin-TFX® administration had no undesirable effect on the reproduction processes in albino rats [Kosmala et al., 1993]. The beneficial effect

of TFX® was, however, observed in pregnant rabbits with primary antiphospholipid syndrome (APS). The syndrome causes disturbances in reproductive processes (pregnancy loss, intrauterine death or growth retardation) by presence of antiphospholipid antibodies which interact with endothelial, trophoblastic and embryonic cells, blood platelets and coagulation factors. These disturbances may lead to changes in aggregation, coagulation and immune response. In an experimental model of APS in pregnant rabbits, intramuscularly (i.m.) injected TFX® increased number of live newborns, limited fetal resorptions and increased survival rate of newborns [Engel-Pietrzak, 2001]. In a study on pregnant sows several nonspecific immunostimulators, such as isoprinosine, 3-hydro-3-methylobutyrate (HMB) or TFX®, administered during pregnancy, were tested for evaluation of colostrums quality. It appeared that all dietary supplements increased IgG level, total protein content and lysozyme activity in the colostrums [Krakowski et al., 2002].

Positive results of TFX® administration were also found in the models of autoimmune diseases. In one study chickens were immunized with myelin basic protein (MBP) for induction of experimental allergic encephalomyelitis (EAE) – an animal model for multiple sclerosis (MS). High levels of specific antimyelin antibodies in IgG class and haptoglobin were found in serum. The treatment with TFX® reduced haptoglobin level but had only a negligible effect on the antibody concentration (humoral response) [Giełdanowski et al., 1987a]. In a more recent study in Lewis rats, with guinea pig spinal cord emulsion-induced EAE, i.m. preventive and therapeutic treatment with a TFX® or its six-peptide fraction, had profound effects on clinical, immunological and ultrastructural changes in the spinal cord. The authors suggest that TFX® or its derivatives may be alternative therapeutic for neurodegenerative diseases such as MS [Zimecki et al., 2010]. The experimental studies on TFX® are summarized in Table 1.

Table 1. The influence of TFX® on immune response in *in vitro* tests and animal models.

Immunological disorders	Experimental model	Therapeutic effects	References
Healthy animals; acute and chronic toxicity tests	Mice, rats, hamsters, guinea pigs, cats, pregnant rats and hamsters; in vitro mutagenic tests (on bacterial cells)	No embryotoxicity, but existence of teratogenic effect, no local and overall toxicity, except high i.v. doses, no mutagenic activity; recommendation for in vivo trials in patients excluding women in the reproductive period	[Ślopek et al., 1980]
	Guinea pigs, rats, rabbits	No adverse effects on physiological parameters and reproduction at doses 8× higher than recommended for clinical use	[Kosmala et al., 1993]
Cytotoxic action of HC, thymectomy, stress	In vitro; mice	Protective effect against the cytotoxic action of HC; restoration of lymphocyte sensitivity in thymectomized mice	[Giełdanowski et al., 1980; Giełdanowski & Ślopek, 1981]
	Mice	Partial restoration of the humoral immune response	[Obmińska-Domoradzka & Debowy, 1996]
	Mice	Restoration of proliferative response of thymocytes to mitogens and thymus weight	[Obmińska-Domoradzka, 1997]
	Mice	Partial reversal of loss of thymocytes and their proliferative response to mitogens	[Obmińska-Mrukowicz & Szczypka, 2005]
	Mice	Counteraction of immune suppression: loss of thymocytes and splenocytes, reduction of the proliferative response of thymocytes to mitogens and IL-1 production by macrophages	[Obmińska--Domoradzka et al., 2002]
Irradiation-damaged spleen lymphocytes from guinea pigs	In vitro	Restoration of splenocytes function and macrophage and granulocyte function produced by lymphocytes	[Kowalczyk-Bronisz & Paegelow, 1986]
Healthy	Dogs, mice	Hemodynamic and hemopoietic activity	[Raberger & Giełdanowski, 1985]
Human and mouse lymphocytes	In vitro	Stimulation of growth of mouse and human T- and B-cell colonies by elicitation of CSF production	[Górski et al., 1981; 1982]
Aging	Mice	Regulation of brain SOD activity and MDA concentration in the brain	[Błońska et al., 1989]

Immunological disorders	Experimental model	Therapeutic effects	References
Trichinella spiralis infection	Mice	Promotion of eradication of larvae from muscles	[Iwanow, 1994]
	Mice	Increasing the number of inflammatory cells in larval capsules in muscle	[Karmańska & Piekarska, 2001]
	Mice	Lowering the numbers of muscular larvae	[Karmańska et al., 2001]
	Mice	Effective increase of the percentage of apoptotic lymphocytes and lowering the parasite load	[Piekarska et al., 2009]
	Mice	Effective regulation of the levels of apoptotic and necrotic lymphocytes	[Piekarska et al., 2010]
	Mice	Potentiation of the phenotypic changes of T cells elicited by infection	[Obmińska--Mrukowicz et al., 2002]
Bacterial (Escherichia coli and Staphylococcus aureus) infections	Mice	Additive effect with antibiotics on the survival time	[Ciebieda et al., 1992]
Neutrophils isolated from diabetic patients	In vitro	Restoration of ingestion and killing ability of neutrophils	[Wysocki et al., 1985]
Neutrophils and T lymphocytes isolated from healthy volunteers	In vitro	Increase of mobility, no effect on chemotactic response	[Smogorzewska et al., 1985]
Neutrophils isolated from healthy volunteers	In vitro	Counteraction of suppression of neutrophil activity (bacterial killing) by PGE2	[Szkaradkiewicz & Kiczka, 1989]
Monocytes isolated from patients with viral hepatitis B	In vitro	Restoration of disturbed monocyte natural cytotoxicity	[Kiczka et al., 1985]
Peripheral blood NK cells isolated from healthy volunteers	In vitro	Stimulatory effect on NK cells killing activity against human leukemia K-562 cells as target cells	[Giełdanowski et al., 1987b]
PBMCs from patients with recurrent HHV-1 infections	In vitro	Increased secretion of IFN-γ and IL-2, but not IL-4 or IL-10	[Hymos et al., 2020]
Primary antiphospholipid syndrome (APS)	Pregnant rabbits	Increased numbers of live newborns, reduction in incidence of fetal resorption, increase in viability and survival rate of newborns	[Engel-Pietrzak, 2001]
Healthy	Pregnant sows	Increase in colostrum's IgG level, total protein content and lysozyme activity	[Krakowski et al., 2002]
Allergic encephalomyelitis (EAE)	Chickens	Decrease in haptoglobin level in serum and slight effect on humoral immunity	[Giełdanowski et al., 1987a]
	Lewis rats	Therapeutic action: clinical, immunological, histological, and ultrastructural improvements	[Zimecki et al., 2010]
Tumour growth	Mice	Prevention of the development of a secondary immune deficit in mice with s.c. transplanted tumours	[Jaszcz et al., 1983]
	Mice	No effect in inhibition of Lewis lung carcinoma in mice, no differences in peritumoral inflammatory reaction vs. untreated mice	[Frąckowiak et al., 1987]
	In vitro	Promotion of differentiation of B lymphocytes isolated from CLL patients (increased proliferative response to mitogen)	[Górski et al., 1983]
	In vitro	Promotion of cytotoxicity of monocytes isolated from patients with alimentary tract cancer	[Kiczka & Szkaradkiewicz, 1986]

Abbreviations: allergic encephalomyelitis (EAE); primary antiphospholipid syndrome (APS); chronic lymphocytic leukaemia (CLL); colony stimulating factor (CSF); human alpha herpesvirus 1 (HHV-1); hydrocortisone (HC); malonyl dialdehyde (MDA); peripheral blood mononuclear cells (PBMCs); prostaglandin E2 (PGE2); superoxide dismutase (SOD)

2.2. *In vitro* studies in human models

In initial studies on mouse and human hemopoiesis TFX® was demonstrated to stimulate growth of mouse and human T- and B-cell colonies by elicitation of colony-stimulating factor (CSF) production. In human tests the activity of TFX® was dependent on initial reactivity of blood donors: it increased low colony formation in low responders and had no effect in high responders. In addition, TFX® stimulated myelopoiesis in mouse and human spleens. The results indicate that TFX® may indirectly stimulate expansion of several cell types, such as T and B cells and neutrophils, by triggering CSF production [Górski et al., 1981]. Furthermore, TFX® is a potent immunomodulator of T-cell-dependent humoral responses in human. Patients with primary glomerulopathies receiving TFX® had enhanced T-cell-dependent humoral responsiveness. Also, in *in vitro* tests with peripheral blood lymphocytes, the preparation stimulated polyclonal antibody synthesis through activation of T-helper cells but decreased antibody production by lymphocytes already involved in high humoral responses (resulting from prior TFX® activation *in vitro* and *in vivo*). TFX® is, therefore, an immunomodulating agent which "senses" individual high or low immune response [Górski et al., 1982]. TFX® also promoted differentiation of B lymphocytes isolated from chronic lymphocytic leukemia patients, as evidenced by an increase to proliferate in response to pokeweed (PWM) mitogen [Górski et al., 1983].

TFX® has also an impact on neutrophil function. A serum-dependent defect in bacterial ingestion and killing, diagnosed in insulin-dependent diabetic patients, can be restored by preincubation of neutrophils with TFX® [Wysocki et al., 1985]. In addition, TFX® was shown to increase mobility of normal T lymphocytes and neutrophils and act as a chemotactic factor [Smogorzewska et al., 1985]. An interesting finding suggested presence of antagonistic actions of exogenous prostaglandin E2 (PGE2), which inhibits bacterial killing by neutrophils, and TFX® was able to counteract this inhibition. The authors postulated a possibility of existence of receptors for thymic factors on neutrophils [Szkaradkiewicz & Kiczka, 1989].

Cytotoxicity of monocytes in certain diseases is lowered. In a study on patients with digestive tract cancer a serum factor was identified, produced also by K562 cell line, which suppressed the cytotoxic action of monocytes from healthy donors. Interestingly, TFX® was able to block production of this factor [Kiczka & Szkaradkiewicz, 1986]. Natural cytotoxicity of blood monocytes is also lower in viral hepatitis patients and is related to liver injury. Also, in this model *in vitro* incubation with TFX® normalized this monocyte function [Kiczka et al., 1985].

Lastly, TFX®, TFX fraction V (TFX V) and synthetic pentapeptide (thymopentin, TP-5) – resembling amino acid sequence in position 32–36 of Goldstein's thymopoietin, were studied for their effects on killing activity of natural killer (NK) cells isolated from human peripheral blood. Best stimulatory dose-dependent effects on NK cell activity were found for TFX V and TP-5. The weaker potency of TFX® could be explained by the fact that fraction V comprised only 15% of the crude TFX® mass [Giełdanowski et al., 1987b]. It was shown that immunotropic activity of thymus extract is localized in fraction V [Giełdanowski & Ślopek, 1981].

The effect on cytokine production by peripheral blood mononuclear cells (PBMCs) derived from adult patients with reactivated human alpha herpesvirus 1 (HHV-1; formerly herpes simplex virus 1, HSV-1) infections was also investigated. PBMC cultures incubated with TFX® (200 μg/ml) demonstrated significantly higher concentrations of IFN-γ and IL-2 but IL-4 and IL-10 production were not enhanced [Hymos et al., 2020]. TFX® *in vitro* studies on human cells are summarized in Table 1.

2.3. *In vivo* studies in human – clinical trials

Respiratory tract infections

The effects of TFX® administration were investigated in children with recurrent respiratory infections. Children (n=117), who had profoundly lower T cell numbers in circulation, were treated with TFX® in combination with levamisole or with other therapeutics, such as Broncho-Vaxom®, PADMA® (an herbal preparation) or subjected to climatotherapy and untreated with immune stimulants. All therapeutic methods had beneficial effect in terms of T-cell number elevation but the best results were obtained in children treated with TFX® and levamisole. However, no correlation was found between this parameter and clinical improvement [Prusek et al., 1987].

TFX® was applied in chronic recurrent upper respiratory tract infections in adults (n=26) resistant to antibiotics, vaccinations and climate treatment. In some patients quantitative or functional defects in T-cell compartment were found. A significant improvement (lower frequency and severity of infections) were registered in 70% of the patients, correlated with correction of T cell number and function [Stankiewicz-Szymczak et al., 1986].

A significant improvement of the clinical state in adult patients (n=162) with chronic spastic bronchitis, who were treated for a long time with corticosteroids, was found following treatment with TFX®. Normalization of granulocyte phagocytic ability was observed, although a positive effect on leukocyte migration

ability *in vitro* and *in vivo* was not noted. In addition, a combined treatment of patients with steroids and TFX® gave better results than the treatment with steroids alone [Matusiewicz et al., 1987; Matusiewicz & Waśniewski, 1988].

Leukopenia, bone marrow insufficiency

In children (n=20) suffering from acute lymphoblastic anemia during remission, TFX® was tested both *in vivo* and *in vitro* for several parameters, such as absolute peripheral blood lymphocyte and T-cell number, skin reactivity to recall antigens and immunoglobulin level. The values of all parameters improved following immunotherapy and the authors concluded that the immune competence of the patients improved, as well as resistance to infections and duration of remission [Traczewska et al., 1985].

The preparation appeared also to have a therapeutic value in 60–70% of cases in patients (n=123) suffering from primary leukopenia in aplastic anemia and myelodysplastic syndrome and secondary leukopenia originated from various iatrogenic causes (antirheumatic agents, antibiotics, cytostatics, irradiation). These effects encompassed accelerated recovery from bone marrow damage and normalization of peripheral blood cell number counts, also in patient resistant to conventional myeloid-reconstituting therapy [Aleksandrowicz et al., 1975; Skotnicki et al., 1984; 1987].

Among diseases characterized by a leukocyte deficit, Hodgkin's disease (malignant lymphogranulomatosis) seems to be particularly predisposed to treatment with TFX® because of leukocyte depletion. The application of TFX® in this category of patients (n=10) led to an increased leukocyte count, enhanced response of T cells in immunological tests and improved hematological tolerance to cytostatics and radiotherapy. TFX® also supported antiviral and antibacterial treatment in patients with co-existing infections [Mariańska-Radziszewska et al., 1975]. Applications of TFX® in patients (n=25) with multiple myeloma, in remission after chemotherapy, improved life quality and prolonged the survival, as well as facilitated chemotherapy by stimulation of myelopoiesis. The similar effect was observed after levamisole applications in other 18 patients [Kraj et al., 1991].

TFX® administered s.c. was, in addition, effective in normalization of leukocyte count in patients (n=18) with persistent lymphopenia of various intensity, resulting from a history of infectious mononucleosis. The total lymphocyte number, B cell, CD4+ and CD8+ T cell number normalization were observed. Isoprinosine therapy, applied in other 15 patients, was also effective [Janeczko, 2001].

Recurrent aphthous stomatitis

Periodic, recurrent oral ulcerations with impaired immune response (local immune complex formation) are specific for recurrent aphthous stomatitis (RAS). TFX® was applied for a long time in 5 patients with RAS with beneficial clinical effects, such as reduction in frequency and severity of the ulcerative lesions and pain [Skotnicki et al., 1984].

Autoimmune disorders

A part of the clinical trials was devoted to therapeutic efficacy of TFX® in autoimmune disorders. In patients with MS quantitative and functional aberrations of the T-lymphocyte and monocyte populations are observed. In a randomized trial including 71 patients with MS, the treatments with TFX® or levamisole followed the therapy with nitrogen mustard in anti-inflammatory doses. Prednisolone was applied in a control group of MS patients. The results showed a significant improvement of neurological state using levamisole or TFX®, but not prednisolone. The authors suggested an indirect therapeutic effect of thymic hormones by immune suppression, via T suppressor cell action, in amelioration of the clinical state [Hertmanowska et al., 1986]. In another, similar study, 20 adult patients were treated i.m. with TFX® or TFX®/dexamethasone for several months. A clinical improvement was observed that was greater in the patients treated only with thymic hormones than in these receiving combined steroid-thymic hormone therapy. The immunological tests showed normalization of the peripheral blood lymphocyte function (response to mitogenic stimulation) and monocyte prostaglandin activity [Dąbrowski et al., 1987].

In a study on 32-year female patient, suffering from systemic lupus erythematosus (SLE) and advanced renal disorders and treated with prednisone without effect, i.m. TFX® was included to the therapeutic regimen. Such a strategy led to diminishment of the symptoms and normalization of the analyzed immunological parameters (decrease in antinuclear antibodies and immunoglobulins, normalization in complement component, T-cell level and delayed skin reaction). As the authors concluded, TFX® may be used as an efficient treatment of the autoimmunological diseases, including SLE [Lasisz et al., 1989].

In a clinical trial on patients (n=7) with Sjögren's syndrome TFX® was applied i.m. for a prolonged time. In all patients the clinical manifestations of the disease and susceptibility to infections were lower. Apart from changes in many immunological indices regarding the circulating blood, in some patients a reversal from negative to positive cutaneous tests for tuberculin and distreptase was registered [Fiedorowicz-Fabrycy et al., 1992].

In addition, in a trial on patients (n=8) with autoimmune hemolytic anemia, after ineffective routine treatment, TFX® administration resulted in disappearance or decrease of serum autoantibody levels [Słomkowski, 1996].

Rheumatoid arthritis (RA) also belongs to the category of diseases with autoimmune etiology. In a clinical trial with juvenile arthritic children (n=10), who had an elevated percentage of CD4+ T lymphocytes and an abnormal CD4+/CD8+ T cell ratio in circulation, the administration of TFX® resulted in correction of the latter parameter in 7 out of 10 patients, associated with improvement of their clinical status [Podwysocka et al., 1989]. The therapeutic effects of TFX® were also evaluated in adult patients suffering from RA. 80% effectiveness in terms of clinical improvement and 40% regarding normalization of biochemical parameters was achieved upon TFX® treatment of adult patients (n=20) suffering from arthritis. These effects were, however, transient [Skotnicki et al., 1986]. Similar results were observed in 20 other patients with RA [Rutowicz, 1987]. In the studies in patients (n=60) with RA, who did not tolerate classical treatments, i.m. TFX® significantly elevated hemoglobin concentration and erythrocyte numbers, as well as decreased parameters of inflammation [Lewandowicz, 1990; 1992]. In another group of RA patients (n=11), demonstrating abnormalities in cellular and humoral immune responses in spite previously treatment with standard therapy, a clinical improvement after s.c. TFX® application was registered [Lasisz et al., 1990].

Another category of diseases, where application of TFX® gave promising therapeutic effects, were chronic skin inflammatory disorders with autoimmunity background, such as psoriasis, scleroderma and dermatomyositis.

TFX® was applied in patients (n=72) with various forms of psoriasis – the disease with inflammatory abnormalities. TFX® administered i.m. was effective in 76.4% of cases, resulting in improvement of the clinical state, including patients with severe form of psoriasis. In some cases, a long-lasting remission was achieved. The improvement of general clinical state and decreased susceptibility to viral and bacterial infections was also registered [Turowski et al., 1987].

Thymotherapy with i.m. TFX® was also effective in 27 adult and teenage patients with scleroderma – the disease affecting skin, muscles, internal organs, connective tissue and blood vessels. Thymic hormones positively influenced typical, numerous symptoms of scleroderma, including healing of chronic ulceration, amelioration of polyarthralgia, esophageal motility disturbances, abdominal pain and cardiorespiratory symptoms in some patients, as well as improved growth and weight gain in several pediatric cases. In some patients the clinical effects were long-lasting. The clinical improvement correlated with the increase of T-cell numbers, together with a drop in gamma globulin concentration [Stępień et al., 1987].

Dermatomyositis is an inflammatory disease of skeletal muscles characterized by weakness of pharyngeal muscles and typical skin rash, and its etiology may have a cell-mediated autoimmune background. The treatment with i.m. TFX®, after discontinuation of the immunosuppressive therapy in 4 patients, led to improvement of all symptoms related to physical impairment, accompanied by normalization of biochemical and immunologic parameters. The results support a hypothesis that dermatomyositis is partially linked to a deficit of T cell homeostatic function [Stasiak, 1983; Zduńczyk et al., 1985].

Viral infections

A series of clinical trials were aimed at evaluation of therapeutic efficacy of TFX® in virally infected patients. In patients (n=60) with acute hepatitis B, i.m. injections of TFX® resulted in correction of biochemical and clinical parameters (decrease of bilirubin and iron levels, shortening of the hospitalization period) [Kiczka et al., 1986].

In the category of patients with chronic active hepatitis B (CAH-B) two clinical trials with long-term administration of TFX® were conducted. In the first trial in 30 patients 60% of cases revealed normalization of alanine transaminase activity and in 50% of the patients a seroconversion from HBeAg+ to anti-HBe was registered. The changes were accompanied by an improvement of general clinical condition, as well as family and professional activity [Juszczyk, 1984; Kiczka et al., 1987].

In the second trial in 21 CAH-B patients an advantageous effect of the clinical course of the disease, with normalization of liver and spleen size and biochemical parameters, was observed [Dąbrowska-Bernstein et al., 1980]. These changes paralleled correction of biochemical and histological parameters in 60–80% of the cases [Cianciara et al., 1984]. In the immunological tests, maturation of T cells upon immunotherapy with TFX® was also noted [Dąbrowski et al., 1980].

In CAH-B both lymphocyte T suppressor (Ts) and NK cell number are enhanced, CD4+/CD8+ cell ratio is lowered and patients demonstrate decreased response to mitogen and allogeneic mixed lymphocyte reaction. In a next clinical trial, the treatment of this category patients (n=21) with TFX® led to correction of these parameters [Zeman et al., 1991].

Similar results were obtained in a still another study on CAH-B patients (n=13) where, in addition to normalization of immunological parameters, serological (seroconversion in the HBeAg+ to anti-HBe), biochemical parameters in liver and clinical improvement were registered [Baj et al., 1991]. These results were confirmed and supplemented by data revealing decrease in NK cell numbers and increase in NK cell activity in other patients (n=18) suffering from CAH-B. The normalization of the biochemical and immunological parameters, seroconversion in HBe system, as well as a complete clinical remission of the disease after two-year treatment with TFX®, were also found. According to the authors, "TFX® has an immunostimulatory action and exerts beneficial effects on the course of CAH-B" [Dworniak et al., 1991].

Although pathological mechanisms involved in liver damage in hepatitis C virus (HCV)-infected patients are not fully explained, the role of the overproduction of reactive oxygen species (ROS) is suggested. At the same time increased ROS production can plays an important role in the suppression of HCV replication. In chronic hepatitis C patients (n=26) a combined treatment of with IFN-α 2a and TFX® resulted in a significant increase of free radical formation by peripheral blood neutrophils, as well as in compensatory increase in serum antioxidant capacity. The biochemical parameters were not different in patients treated with IFN-α 2a alone and with IFN-α 2a/TFX® [Jabłonowska et al., 2005].

In a large study in women (n=130) with human papillomavirus (HPV) infection of the uterine cervix, a combined treatment with IFN-β and TFX® cured 90% of patients versus 88% treated with IFN-β alone. The best protection (94.2%) was obtained in patients receiving 13-cis-retinoic acid (provitamin A) in conjunction with IFN-β [Markowska et al., 1994].

The therapeutic efficacy of TFX® was also reported in patients (n=28) with acute herpes zoster, leading to amelioration of the clinical symptoms (reduction in number and size of vesicles and duration of pain) and shortening the course of the disease [Skotnicki unpublished data, see Skotnicki, 1989a].

TFX® may also prevent or ameliorate clinical symptoms of HHV-1 infection in patients with recurrent facial herpes simplex (herpes labialis). In 7 out of 8 adult patients with history of herpes labialis TFX® applications significantly improved recurrence rate and clinical symptoms of the infection. In 2 females TFX® prevented also reactivation and ameliorated clinical course of facial and genital herpes simplex infection [Skotnicki unpublished data, see Skotnicki, 1989a]. Efficacy of TFX® treatment in patients with reactivated HHV-1 infection was also proved in the latest study in 50 women. Following 2-month s.c. TFX® treatment the content of B cells and T cell subsets, as well as expression of proapoptotic PD-1 and PD-L1 markers, were analyzed. The thymomodulin treatment resulted in increase of T CD3+ and B CD19+ cell content and decrease of T and B cell numbers bearing the pro-apoptotic markers. Production of IL-2 and IFN-γ by recovered PBMCs, after *in vitro* stimulation with TFX®, was also increased. In addition, the treatment with TFX® resulted in absence of reinfection [Hymos et al., 2020].

Anticancer therapy

The utility of TFX® in anticancer therapy was also a subject of several investigations. Positive effects of TFX® treatment were described in patients (n=20) with chronic lymphocytic leukemia (CLL). In 70% of leukemia patients the treatment with TFX® reduced the total lymphocyte count, accompanied by increase of T cell, reticulocyte, immunoglobulin and hemoglobin levels, as well as immune reactivity, measured as skin sensitivity to tuberculin [Skotnicki et al., 1978; Wazewska-Czyżewska et al., 1976]. As mentioned above, TFX® was also effective in 10 patients with advanced Hodgkin's disease by improving T-cell-dependent immunity, hematological tolerance to chemo- and radiotherapy and defense against mycobacterial and viral infections [Mariańska-Radziszewska et al., 1975].

Similar effects of TFX® treatment were found in patients (n=50) with inoperable colorectal cancer, in whom enhancement of the cellular immunity was registered, together with increased granulocyte and lymphocyte content, clinical improvement and increased survival time. The observed prolongation of survival may be attributable to mobilization by thymic hormones of local immune reactions against tumor cells, since the characteristic microscopic changes with focal calcification and tumor necrosis were described [Cubulski et al., 1976; Turowski et al., 1976; Urban et al., 1977]. Also, in a case report, a regression of metastatic gastric cancer after long term TFX® treatment was confirmed by autopsy [Hryniewiecki & Kiczka, 1985].

TFX® may also be used as an adjunct with postoperative radiotherapy. This procedure in breast cancer patients (n=20) changed the composition of peripheral blood lymphocytes (CD2, CD3, CD4, CD8 and CDDR positive cells). The postoperative treatment of patients with radiotherapy and immunotherapy with TFX® resulted in a lower decrease of CD4 and CDDR positive cell content, in comparison to a control group treated with radiotherapy only [Misković et al., 1994].

According to the authors of a review, which summarizes 15-year study in 457 patients with surgical can-

cers of gastrointestinal tract and breast, a diminished incidence of postoperative complications and shorter healing time following TFX® treatment, was of special importance [Cybulski & Turowski, 1987].

TFX® was also found effective in prolongation of the life span of patients with primary lung cancer. In patients (n=41) with less advanced carcinoma, admitted to radiotherapy, the preparation extended mean 6 and 12-month survival rates from 31% to 69% and from 7% to 38%, respectively. The 6-month survival time in patients (n=53), with advanced disseminated cancer process and excluded from conventional therapy, was 42% in TFX® group versus 7% in the control group. These effects were clearly associated with reconstitution of the immune function, as measured by increased delayed hypersensitivity reaction. Hematological and immunological tolerance to irradiation was higher in patients with adjunct immunotherapy with TFX® [Krzysko & Słowik-Garyelska, 1981; Słowik-Gabryelska & Krzysko, 1980; Żeromski et al., 1976].

Allografts

In a trial involving patients (n=40) with chronic kidney graft rejection, resistant to classical immunosuppression, TFX® treatment had an advantageous effect on kidney function and time of organ rejection [Górski et al., 1985].

Healthy individuals

Of interest, TFX® treatment may also change immunologic parameters of healthy volunteers subjected to extreme physical effort. It appeared that a short stimulation of cyclists with TFX® corrected parameters altered by the intensive physical exercise, such as decreased response of peripheral lymphocytes to mitogens, decreased CD4+/ CD8+ cell ratio and increased mononuclear cells number with HLA DR and CD71 antigens [Tchórzewski et al., 1992]. The clinical studies on TFX® are summarized in Table 2.

Table 2. Clinical trial with TFX® administration.

Diagnosis	Short description of study	Clinical effects	References
Respiratory tract infections	Children with recurrent respiratory infections; n=117	Beneficial effect on T-cell number, no correlation with clinical improvement	Prusek et al., 1987]
	Adults with chronic recurrent upper respiratory tract infections; n=26	Lower frequency and severity of infections in 70% of the patients correlated with correction of T cell number and function	[Stankiewicz-Szymczak et al., 1986]
	Adults with chronic spastic bronchitis; n=162	Improvement of the clinical state, normalization of granulocyte phagocytic ability	[Matusiewicz et al., 1987; Matusiewicz & Waśniewski, 1988].
Leukopenia, bone marrow insufficiency	Children with acute lymphoblastic anemia during remission; n=20	Improvement of the immune competence, resistance to infections and duration of remission	[Traczewska et al., 1985]
	Patients with aplastic anemia, myelodysplastic syndrome and secondary iatrogenic leukopenia; n=123	Accelerated recovery from bone marrow damage and normalization of peripheral blood cell number counts	[Aleksandrowicz et al., 1975; Skotnicki et al., 1984, 1987]
	Patients with Hodgkin's disease (malignant lymphogranulomatosis); n=10	Increased leukocyte count and response of T cells in immunological tests, improvement of hematological tolerance on cytostatics and radiotherapy	[Mariańska-Radziszewska et al., 1975]
	Patients with multiple myeloma in remission; n=25	Improvement of life quality and prolongation of the survival, facilitation of chemotherapy by stimulation of myelopoiesis	[Kraj et al., 1991]
	Patients with persistent lymphopenia resulting from infectious mononucleosis; n=18	Normalization of the total lymphocytes, B cell, CD4 and CD8 T cells number	[Janeczko, 2001]
Recurrent aphthous stomatitis (RAS)	Patients with RAS; n=5	Clinical improvement: reduction in frequency and severity of the ulcerative lesions and pain	[Skotnicki et al., 1984]

Diagnosis	Short description of study	Clinical effects	References
Autoimmune disorders	Patients with MS after therapy with nitrogen mustard; n=71	Improvement of neurological state, possible immune suppression through T suppressor cells activation	[Hertmanowska et al., 1986]
	Patients with MS; n=20	Clinical improvement, normalization of peripheral blood lymphocyte and monocyte function	[Dąbrowski et al., 1987]
	32-year female patient suffered from SLE (case report)	Clinical improvement, normalization of immunological parameters	[Lasisz et al., 1989]
	Patients with Sjögren's syndrome; n=7	Clinical improvement, normalization of immunological parameters, lowering susceptibility to infections	[Fiedorowicz-Fabrycy et al., 1992]
	Patients with autoimmune hemolytic anemia; n=8	Disappearance or lowering serum autoantibody level	[Słomkowski, 1996]
	Children with juvenile arthritic; n=10	Clinical improvement, correction of CD4+ T cell level and CD4/CD8 T cell ratio	[Podwysocka et al., 1989]
	Adult patients with RA; n=20	Clinical improvement, normalization of biochemical parameters	[Skotnicki et al., 1986]
	Adult patients with RA; n=20	Clinical improvement, normalization of biochemical parameters	[Rutowicz, 1987]
	Adult patients with RA who not tolerated classical treatments; n=60	Elevation of hemoglobin concentration and erythrocyte numbers, alleviation of inflammation parameters	[Lewandowicz, 1990; 1992]
	Adult patients with RA previously ineffectively treated with standard therapy; n=11	Clinical improvement	[Lasisz et al., 1990]
	Patients with psoriasis, also severe form; n=72	Clinical improvement; lowering susceptibility to viral and bacterial infections	[Turowski et al., 1987]
	Adult and teenage patients with scleroderma; n=27	Amelioration of typical symptoms of scleroderma, normalization of immunological parameters	[Stępień et al., 1987]
	Patients with dermatomyositis after immunosuppressive therapy; n=4	Improvement of all symptoms, normalization of biochemical and immunological parameters	[Stasiak, 1983; Zduńczyk et al., 1985]
Viral infections	Patients with acute hepatitis B; n=60	Improvement of biochemical and clinical parameters	[Kiczka et al., 1986]
	Patients with CAH-B; n=30	Improvement of biochemical and clinical parameters; seroconversion	[Juszczyk, 1984; Kiczka et al., 1987]
	Patients with CAH-B; n=21	Normalization of biochemical and histological parameters; maturation of T cells	[Cianciara et al., 1984 ; Dąbrowska-Bernstein et al., 1980; Dąbrowski et al., 1980]
	Patients with CAH-B; n=21	Beneficial clinical effect and immunoregulatory action	[Zeman et al., 1991]
	Patients with CAH-B; n=13	Clinical improvement, normalization of biochemical and immunological parameters; seroconversion	[Baj et al., 1991]
	Patients with CAH-B; n=18	Clinical improvement, normalization of biochemical and immunological parameters; seroconversion	[Dworniak et al., 1991]
	Patients with chronic hepatitis C treated with IFN-α 2a; n=26	Increase in formation of ROS by blood neutrophils, increase in serum antioxidant capacity	[Jabłonowska et al., 2005]

Diagnosis	Short description of study	Clinical effects	References
Viral infections	Women with HPV infection treated with IFN-β; n=130	Protection of 90% patients vs 88% treated with IFN-β alone	[Markowska et al., 1994]
	Patients with acute herpes zoster; n=28	Clinical improvement (reduction of new lesions and duration of pain, acceleration of healing)	[Skotnicki unpublished data, see Skotnicki et al., 1989]
	Patients with recurrent herpes labialis; n=8	Clinical improvement (no or lower recurrences)	[Skotnicki unpublished data, see Skotnicki et al., 1989]
	Patients with recurrent herpes labialis; n=50	Clinical improvement (no reactivation of HHV-1 infection), increase of T CD3+ and B CD19+ cell content, reduction of inhibitory marker (PD-1 and PD-L1) expression on T and B cells	[Hymos et al., 2020]
Anticancer therapy	Patients with CLL; n=20	Normalization of immunological parameters	[Wazewska-Czyżewska et al., 1976; Skotnicki et al., 1978]
	Patients with advanced Hodgkin's disease; n=10	Normalization of immunological parameters, increase of defense against infections	[Mariańska--Radziszewska et al., 1975]
	Patients with inoperable colorectal cancer; n=50	Clinical improvement, prolongation of survival	[Cubulski et al., 1976 ; Turowski et al., 1976; Urban et al., 1977]
	Patient with metastatic gastric cancer (case report)	Regression of cancer	[Hryniewiecki & Kiczka, 1985]
	Patients with breast cancer treated with radiotherapy; n=20	Normalization of immunological parameters	[Misković et al., 1994]
	Patients with primary lung cancer; n=94	Prolongation of survival, reconstitution of immune function	[Krzysko & Słowik--Garyelska, 1981; Słowik-Gabryelska & Krzysko, 1980; Żeromski et al.,1976]
Allografts	Patients with chronic kidney graft rejection; n=40	Improvement of kidney function and prolongation time of organ rejection	[Górski et al., 1985]
Healthy individuals	Healthy volunteers subjected to extreme physical effort	Normalization of immunological parameters	[Tchórzewski et al., 1992]

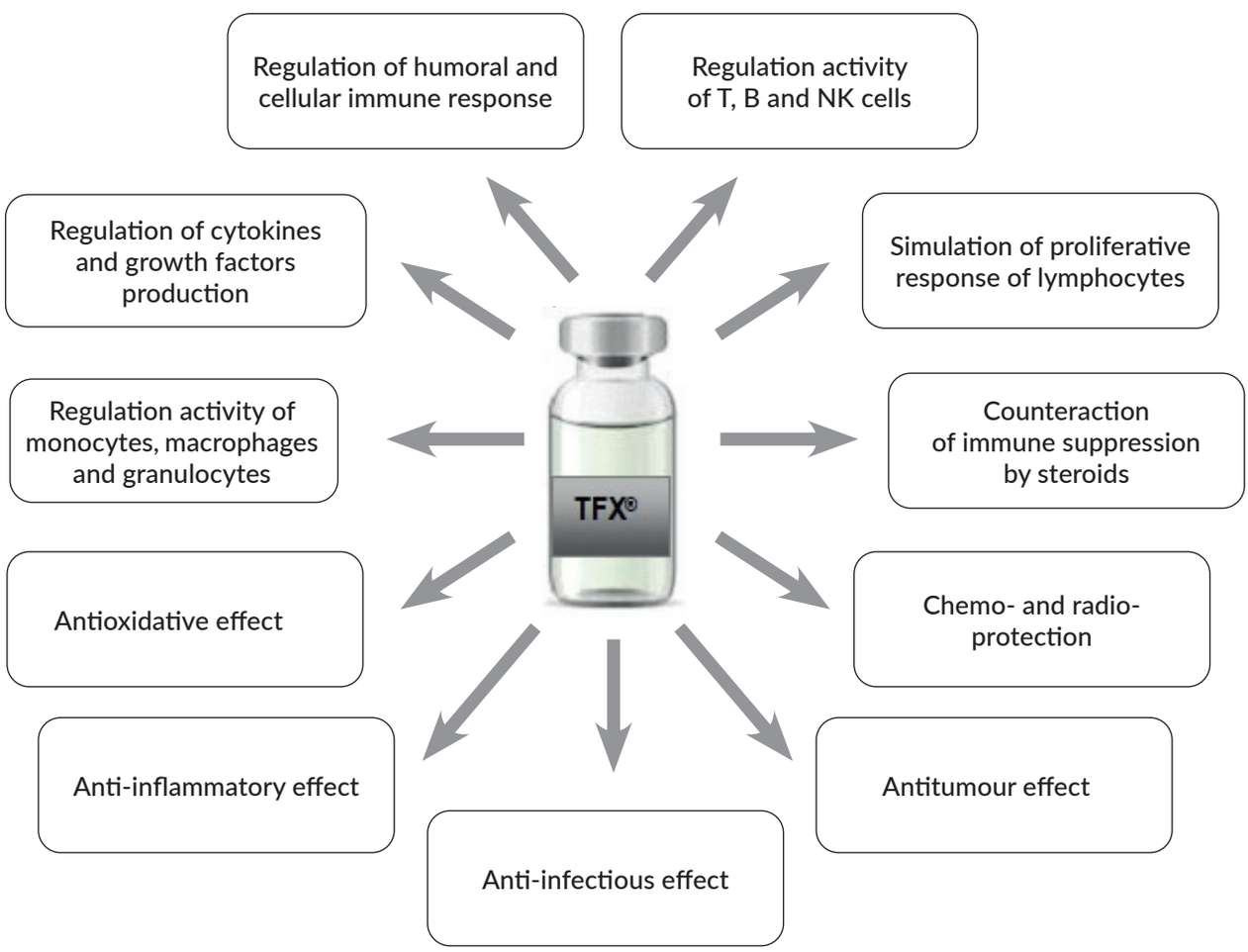
Abbreviations: chronic active hepatitis B (CAH-B); chronic lymphocytic leukaemia (CLL); human papillomavirus (HPV); multiple sclerosis (MS); rheumatoid arthritis (RA); recurrent aphthous stomatitis (RAS); systemic lupus erythematosus (SLE)

3. Conclusions

The presented data, collected from extensive studies on regulatory activities of thymic hormones/TFX® in animal and human models, provided sufficient evidence for therapeutic utility of this preparation as a supportive and preventive approach in various clinical states with immune, particularly T-cell-mediated disturbances. Considering its lack of systemic and organ toxicity (no side effects) and facility in applications, TFX® may be an important remedy for both immune prophylaxis as well as immune intervention. The mechanisms of TFX® activity involve overall immune correction, i.e. reconstitution of the immune system function. As agonists of toll-like receptors (TLR) thymic hormones/TFX® activate dendritic cell (DC) and T-helper (CD4+) cells for secretion of cytokines such as IL-2, IFN-α, IL-7, IL-15 and TNF-α. The cytokines, in turn, activate NK cells, macrophages and cytotoxic T (CD8+) lymphocytes increasing their antiinfectious and antitumor activities. In parallel, thymic hormones interact with regulatory T (CD4+/CD25+FoxP3+) lymphocytes, inducing IL-10 and TGF-β, that lowers excessive reactivity of immune cells and restricts inflammatory processes. Thus, thymotherapy provides natural, physiologic immunoregulatory action, by restoring homeostasis of the immune system, resulting in therapy of immune deficiencies, infections, neoplastic diseases, allergy and autoimmunity [Skotnicki 2019]. The pleiotropic immune properties of TFX® are presented in Fig. 1.

It also appears that there is no particular need to isolate specific peptides from TFX® preparation since an additive effect of several active peptides may account for its overall biological property. It is, however, apparent that the molecular mechanism of action of TFX® needs to be clarified applying modern molecular immunogenetic techniques and the cellular mechanism of action requires phenotypic determination of regulatory T and B cells presumably generated by TFX® in pathological circumstances.

Fig. 1. Pleiotropic immune properties of TFX®.



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