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Calf thymus extract attenuates severity of experimental encephalomyelitis in Lewis rats

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Abstract

The aim of this study was to evaluate the efficacy of treatment of Lewis rats with calf thymus extract (TFX®) and its six-peptide fraction on the course of experimental allergic encephalomyelitis (EAE). Interferon-β served as a reference drug. We found that intramuscular administration of the thymus extract fraction significantly reduced clinical, immunological, histological, and ultrastructural alterations inherent in the disease. We suggest that TFX® or TFX®-derived fractions have potential as therapeutics in treatment of neurodegenerative diseases such as multiple sclerosis.

Key words: EAE, Lewis rats, TFX®, IFN-β.

Introduction

Multiple sclerosis (MS) is a neurodegenerative disease [21], mediated by autoreactive Th1 type cells and characterized by inflammatory cell infiltrations and areas of demyelination of axon sheaths. Nevertheless, MS is not entirely consistent with the CD4-Th1 model as proposed by Lassman and Rnasohoff [15]. Recently, an important role for pro-inflammatory Th17 cells in the pathogenesis of MS was also documented [19]. Among several laboratory models relevant to MS, applying rodents and primates, the model using susceptible strains of rats is still commonly used [29,30].

Therapeutic strategies in MS are diverse and include, among others, application of steroids, cytokines and other compounds, autoantigen, transplantation of bone marrow cells and activation of regulatory T cells [17,18,28,31]. Interferon-β remains, despite its limitations [4], a first-line therapy in MS [23,25].

The frequency of autoimmune disorders increases due to aging, environmental pollution and use of immune suppressors, resulting in thymus involution, diminution of the T-cell repertoire and expansion of autoreactive T-cell clones [11,26]. Thymus hormones [10] could be, therefore, an attractive alternative to glucocorticoids [17] or other immunosuppressors [13] for treatment of autoimmune diseases.

A semi-purified calf thymus extract (TFX®) was studied for its immunoregulatory and therapeutic properties a few decades ago. TFX® was shown, among others, to induce thymocyte differentiation [7], activate natural killer cells *in vitro* [9], exert anti-inflammatory properties [8], enhance T- and B-cell colony formation, and stimulate myelopoiesis in mice

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and humans [12]. The preparation was a subject of numerous clinical trials [27] encompassing treatment of various dysfunctions of the immune system and showing a benefit of long-term immunotherapy with TFX®.

Reports on the effect of thymic factors on experimental encephalomyelitis in rodents are scanty. Kato and Nakamura [14] found that a synthetic serum thymic factor suppressed clinical symptoms of EAE in Hartley guinea pigs and Lewis rats, and the inhibitory action of thymic factor was due to an increase of suppressor OX8+ cells.

The aim of this work was to evaluate the efficacy of TFX® and reference preparations, such as interferon β and vasoactive intestinal peptide (VIP), on the course of EAE in susceptible Lewis rats, by determination of the clinical score and cytokine production, as well as performance of histological, cytochemical and ultrastructural analyses of respective organs.

Material and methods

Animals

Female Lewis rats, susceptible to EAE, weighing 195 g on average, were purchased from the Medical University of Warsaw. The animals were fed a standard, granulated food and tap water *ad libitum*. The local ethics committee approved the study.

Reagents

All reagents were purchased from Sigma-Aldrich unless otherwise stated. Other commercially available reagents: OptEIA™ for rat IL-10, TNF-α, IFN-γ, and human TGF-β1 were obtained from BD Biosciences Pharmingen; interferon-β-1b (Betaferon®) was purchased from Schering AG; antibodies against IL-6 and TNF-α were provided by Abcam; anti-myelin basic protein antibodies and EnVision™ Systems were obtained from Dako. Synthetic vasoactive intestinal peptide (VIP) was purchased from Sigma-Aldrich.

Elicitation of EAE

The EAE reaction was induced as described in [33]. Briefly, 0.1 ml of an emulsion consisting of 2 mg of guinea pig spinal cord in phosphate buffered saline (PBS), and Freund's incomplete adjuvant (Difco) admixed with 4 mg/ml of *Mycobacterium tuberculosis* (Difco) was injected in each hind foot of the experimental rats.

Clinical evaluation of EAE

The rats were evaluated every day for EAE symptoms and weighed from the day of immunization. Clinical signs of the disease were graded, as reported elsewhere [33], using the following criteria: 1 – lack of tone in the tail, +2 – hind limb hypotonia, +3 – partial hind limb paralysis, and +4 – complete hind limb paralysis and incontinence.

Isolation and purification of TFX® and its fractions

TFX® was isolated from calf thymuses as originally described (patent No. 108 714). A standard TFX® preparation consists of 15 peptides. TFX® was also subjected to chromatography on a Sephadex 25 fine column. The studied fractions contained peptides of M.W. < 5 kD > 2 kD. In fraction II, 6 peptides could be identified by means of polyacrylamide isoelectric focusing.

Administration of preparations

All preparations were administered intramuscularly, consistent with the therapeutic way of treatment [27] in a volume of 0.2 ml. In the preliminary experiments TFX® was given in daily doses, from the day of immunization until day 29. The optimal daily dose of TFX® (200 µg/rat) was established in preliminary experiments as 1/50 of the human dose (10 mg). The dose of TFX® fractions (I, II and III) was 140 µg/rat/day and the preparations were given from the day of immunization until day 29. In the next part of the investigation the therapeutic effect of TFX® fraction II was analysed. The dose of TFX® fraction II was 140 µg/rat/day and the preparation was given from day 11 to 21. The dose of interferon β (2.5 µg/rat/day – 80 000 units; i.m.) was calculated from a human dose; the cytokine was administered for 5 consecutive days beginning on day 11 after immunization. Vasoactive intestinal peptide (VIP) was given at 2.5 µg/rat doses beginning on day 11 for 5 consecutive days. Control rats (not subjected to therapy) received daily injections of 0.9% NaCl.

Isolation of blood and lymph nodes

The rats were subjected to general anaesthesia and bled by heart puncture. Blood was allowed to clot and was centrifuged at 300 × g to separate serum,

aliquoted and stored at –20°C until cytokine determination. Inguinal lymph nodes were isolated and homogenized to obtain a single cell suspension. The cells were then resuspended in a culture medium consisting of RPMI-1640 medium, 10% fetal calf serum, L-glutamine, sodium pyruvate, 2-mercaptoethanol and antibiotics.

Induction of cytokines and determination of cytokine activities

Lymph node cell cultures (2×10^6 cells/ml) were exposed to a guinea pig spinal cord lysate (100 µg/ml). Supernatants were harvested after 48 h for cytokine measurement. The supernatants were stored at –20°C until cytokine determination using the OptEIA™ kits according to the manufacturer's instructions.

Immunohistochemistry

Thoracic parts of the spinal cord were isolated immediately after sacrifice of animals. Each specimen was cut into two 0.3 cm-long pieces and fixed in 10% buffered formalin and embedded in paraffin. Sections 3 µm thick were prepared in Zeiss Microm HM 340E and placed on histological slides. Then the samples were deparaffinized and dehydrated applying xylene and graded alcohol series. The sections were stained using immunoperoxidase cell staining kits. Antibodies against IL-6 and TNF-α were used to estimate the intensity of the inflammatory response within the examined tissues. In addition, anti-myelin basic protein antibodies were applied for visualization of the myelin membranes. Heat-induced antigen retrieval was performed by incubation of the slides in Tris/EDTA buffer (pH 9.0) for 20 min. The endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 5 min. Then slides were washed with TBS for 5 min and incubated with primary antibody for 1 h at room temperature, followed by three 5-min washes with TBS. The detection was performed with EnVision™ Systems. Then the sections were counterstained with Mayer's haematoxylin and observed by means of light microscopy (Axio Imager A1).

Histology

Spinal cords were fixed in 4.0% formalin solution for 48 h, washed for 24 h, dehydrated in alcohol and finally embedded in paraffin. The paraffin sections were sliced in a Micron HM310 microtome into 6 µm sections and stained with haematoxylin and eosin (H&E). The histological analysis was performed in a light microscope, Nikon Eclipse 801.

Ultrastructural analysis

Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After several rinses in the same buffer the material was post-fixed for 2 h in 2% osmium tetroxide in the buffer. Following dehydration in acetone series (30-100%), the material was embedded in Epon 812. Ultrathin 70 nm sections were contrasted with uranyl acetate and lead citrate and examined in transmission electron microscopy (Tesla BS500).

Statistics

Each experimental group consisted of 8 mice. Results are expressed as the mean or the median (for parametric and nonparametric data, respectively). Statistical differences between groups were evaluated using parametric analysis (ANOVA with post-hoc Tukey's test) or nonparametric analysis by Mann-Whitney *U* test or Kruskal-Wallis test (if assumptions for the parametric test were not met) with *P*-value of < 0.05 being considered significant.

Results

Protective effects of the standard TFX® preparation on development of EAE: evaluation of clinical symptoms

The aim of the first experiment was to establish whether TFX® administered daily from the day of immunization for 29 days (preventive application) at the dose of 200 µg per animal would influence the clinical symptoms of EAE. The results are shown in Fig. 1 and Table I. The reaction developed typically with peak intensity on day 15, decreasing thereafter (Fig. 1A). The treatment of rats with TFX® led from day 17 to a marked amelioration of the clinical symptoms. The treatment with TFX® also resulted in a better weight gain by rats during days 20-29 following immunization (Fig. 1B).

Since TFX® is composed of 15 peptides an attempt was undertaken to isolate a fraction consisting of fewer peptides, still preserving the activity of the whole preparation. TFX® was subjected to chroma-

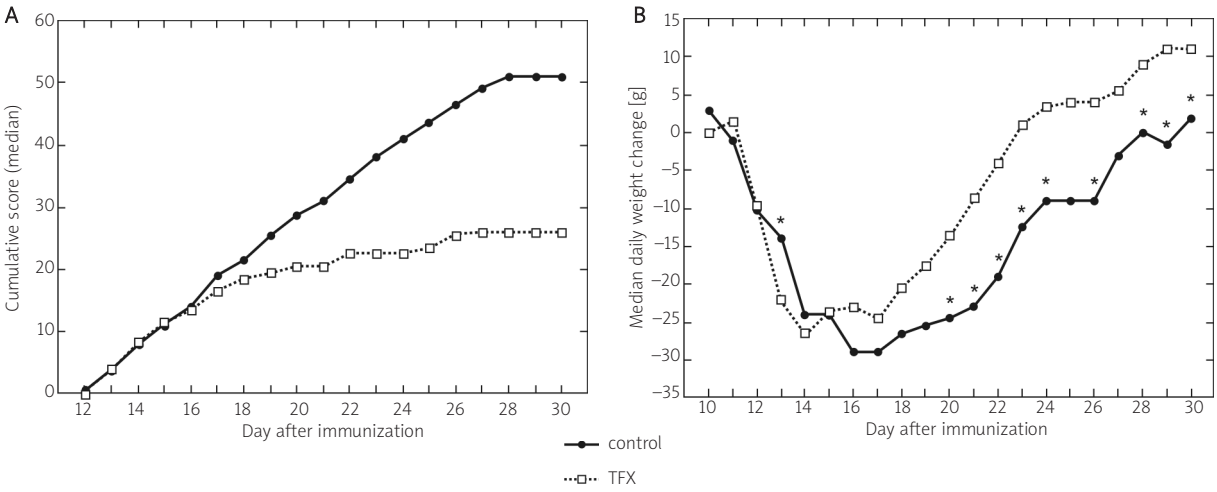

Fig. 1A-B. Protective activity of TFX®. **A)** Effects of TFX® on the clinical score in EAE rats. TFX was administered daily from the day of immunization for 29 days at the dose of 200 µg/rat/day. The cumulative score for every individual was calculated by adding all given points in the testing time frame. The median daily cumulative score from 8 rats per group was presented (for statistical analysis see Table I). **B)** Effect of TFX® on weight of rats during EAE reaction. Daily weight change of every individual was calculated by subtracting weight on the ninth day after immunization (the last day with weight gain before first clinical sign of EAE). The median daily weight change from 8 rats per group was presented. Weight loss during EAE as compared to the control group was analysed using the Mann-Whitney *U* test. *$P < 0.05$.

Table I. Clinical score of EAE. The median score for each animal over the clinical period of the disease was calculated (day 11-30 after immunization) and the cumulative score for every individual was calculated by adding all given points in the testing time frame (day 0-30 after immunization). The median score and the median cumulative score from 8 rats per group were presented. Statistical differences between groups were analysed using

Fig. No.	Treatment	Median Score (time frame of clinical disease course)	Median Cumulative Score	Median No. of Days with Score above 2
(Fig. 1A)*	Control	3.00	51.00	11.00
	TFX®	1.00 ($P = 0.0117$)	26.00 ($P = 0.0070$)	4.00 ($P = 0.0074$)
(Fig. 2)#	Control	2.50	46.50	10.50
	Fraction I of TFX®	0.00 ($P = 0.0500$)	20.75 ($P = 0.0410$)	4.00 ($P = 0.0240$)
	Fraction II of TFX®	0.00 ($P = 0.0009$)	15.00 ($P = 0.0000$)	2.50 ($P = 0.0002$)
	Fraction III of TFX®	2.00 NS	32.00 NS	6.00 NS
(Fig. 3)#	Control	3.00	57.00	13.50
	Fraction II of TFX®	0.00 ($P = 0.0057$)	19.25 ($P = 0.0020$)	3.50 ($P = 0.0091$)
	VIP	0.00 ($P = 0.0120$)	22.75 ($P = 0.0349$)	5.00 NS
	IFN-β	2.00 NS	32.50 NS	6.00 NS

**Mann-Whitney test*
#Kruskal-Wallis test (versus Control)

tography as described in the experimental section and three fractions were obtained. These fractions were tested in the EAE model (Fig. 2 and Table I). The results clearly indicated that the bulk of activity resided in fraction II consisting of 6 peptides. Fraction I was less active and fraction III demonstrated negligible activity. Therefore, fraction II was used for further experiments.

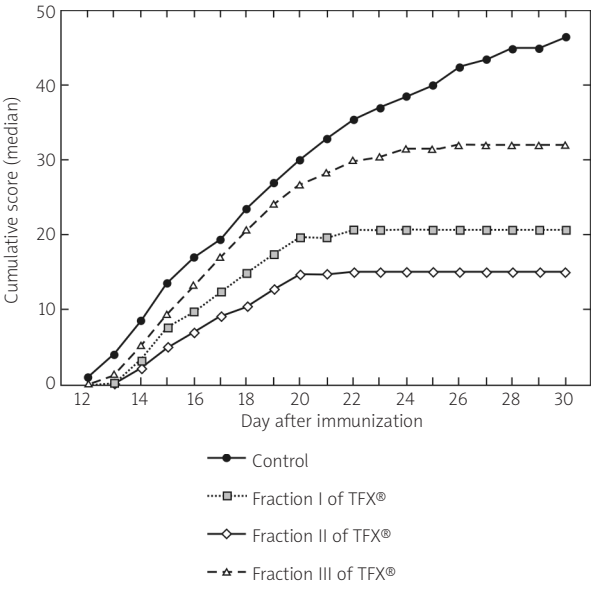

Fig. 2. Protective effects of TFX® fractions on the clinical score of EAE rats. TFX fractions were administered daily from the day of immunization for 29 days at the dose of 140 µg/rat. The cumulative score for every individual was calculated by adding all given points in the testing time frame. The median daily cumulative score from 8 rats per group was presented; for statistical analysis see Table I.

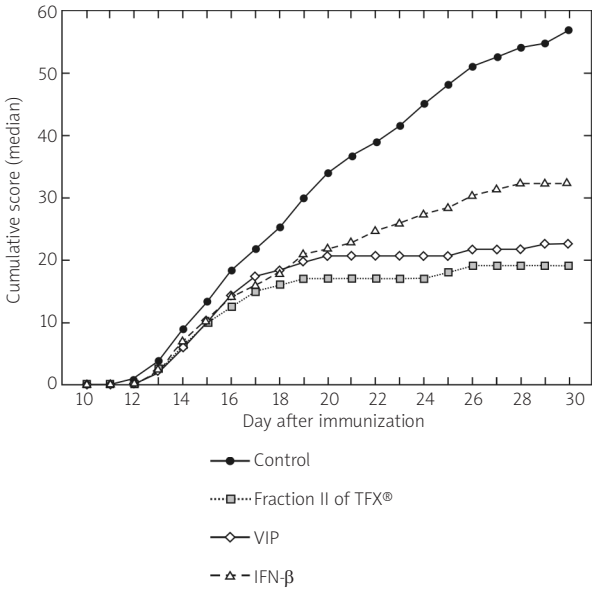

Fig. 3. Effects of the therapeutic administration of TFX® fraction II, IFN-β and vasoactive peptide (VIP) on the clinical score of EAE rats. TFX® fraction II was administered at the dose of 140 µg/rat from day 11 to 21 of the experiment, IFN-β at the dose of 2.5 µg/rat (80 000 units), daily from day 11 to 15, and VIP at the dose of 2.5 µg/rat, daily from day 11 to 15. All preparations were administered intramuscularly. The cumulative score for every individual was calculated by adding all given points in the testing time frame. The median daily cumulative score from 8 rats per group was presented; for statistical analysis see Table I.

Comparison of the therapeutic effects of TFX® fraction II, IFN-β and vasoactive peptide (VIP) on the clinical score in EAE rats

Next, we wished to compare the therapeutic efficacy of TFX® fraction II with interferon β, routinely used for treatment of sclerosis multiplex [23], and vasoactive intestinal peptide, also reported to inhibit EAE [6,16]. The preparations were administered from day 11 (first symptoms of EAE), as indicated in the experimental section. The results showed that all studied preparations inhibited the intensity of clinical changes (Fig. 3 and Table I). However, statistically significant inhibition of EAE was registered only with TFX® and VIP.

Therapeutic effects of TFX® fraction II and IFN-β on selected immunological parameters of EAE rats

For a more complete evaluation of the therapeutic effect of TFX® fraction II, as compared with IFN-β, we extended the investigations to determination of additional immunological parameters as well as histological analysis of the spinal cord. Circulating blood, inguinal lymph nodes and spinal cords were isolated from rats on day 17 following immunization. The clinical scores of rats in TFX® fraction II and IFN-β groups were similar to those shown in Fig. 3, and are therefore not shown. As expected, the cell numbers in the draining lymph nodes were significantly higher in untreated rats as compared to TFX® fraction II and IFN-β-treated rats (Fig. 4). Similarly (Fig. 5), the numbers of circulating leukocytes were significantly lower in these groups as compared to untreated control rats.

The effects of treatment of EAE rats with TFX® fraction II and IFN-β on antigen-specific induction of cytokines in the cultures of lymph node cells are shown in Fig. 6. Marked differences in cytokine production were registered in the case of IL-10 (Fig. 6A),

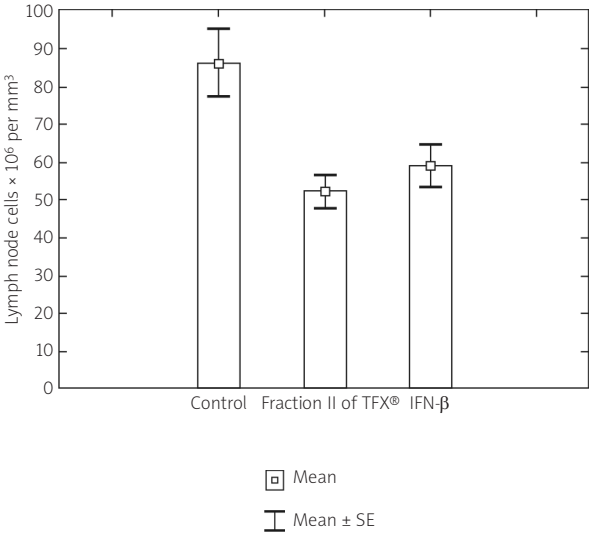

Fig. 4. Effects of TFX® fraction II and IFN-β on the cell numbers of inguinal lymph nodes. The preparations were administered as described in Fig. 3. Statistical differences between groups were analysed using the ANOVA and Tukey's test: Control versus fraction II of TFX® (P = 0.0065); Control versus IFN-β (P = 0.0265).

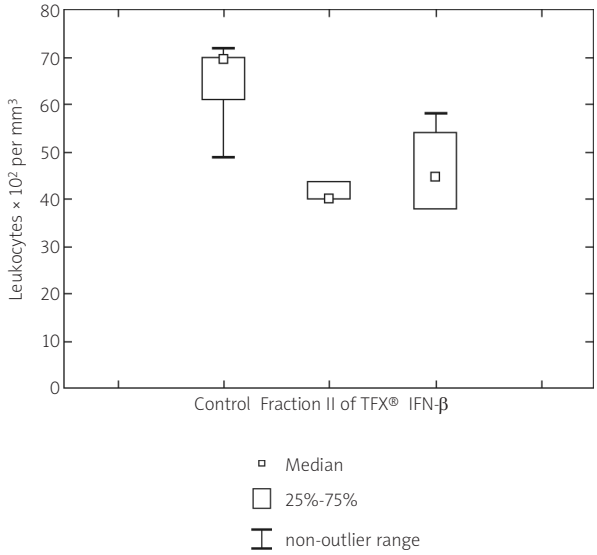

Fig. 5. Effects of TFX® fraction II and IFN-β on the numbers of circulating leukocytes. The preparations were administered as described in Fig. 3. Statistical differences between groups were analysed using the Kruskal-Wallis test and post-hoc Dunn's test: Control versus fraction II of TFX® (P = 0.0283); Control versus IFN-β (P = 0.0283).

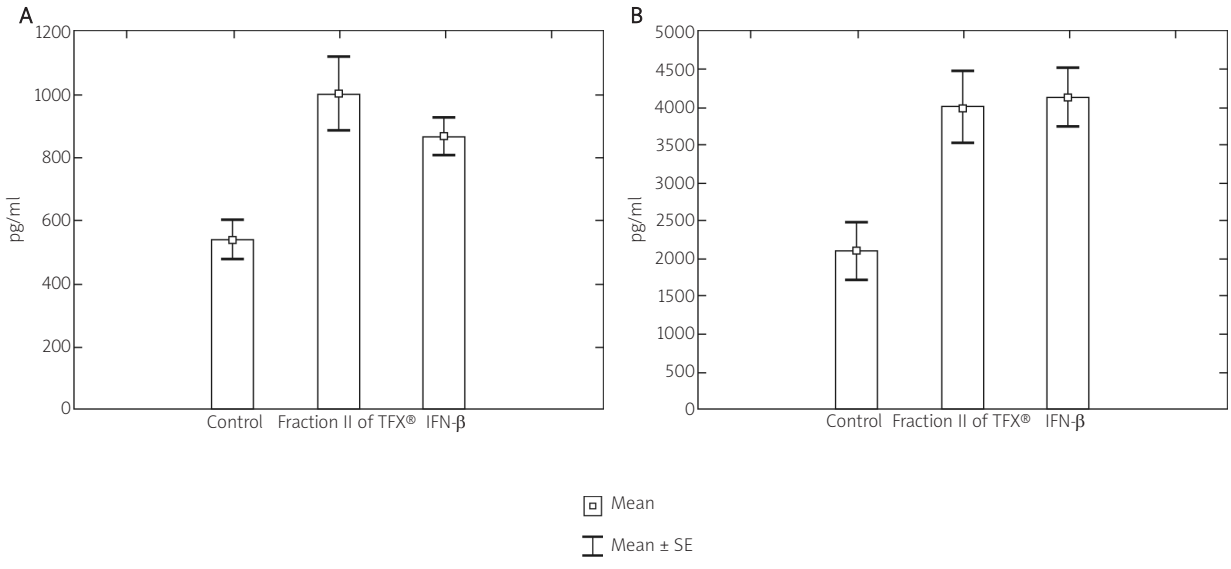

Fig. 6A-B. Effect of TFX® fraction II and IFN-β on cytokine production by lymph node cell cultures stimulated by spinal cord extract. The preparations were administered as described in Fig. 3. **A)** IL-10 production. Statistical differences between groups were analysed using ANOVA and Tukey's test: Control versus fraction II of TFX® (P = 0.0034); Control versus IFN-β (P = 0.0344). **B)** IFN-γ production. Statistical differences between groups were analysed using ANOVA and Tukey's test: Control versus fraction II of TFX® (P = 0.0201); Control versus IFN-β (P = 0.0071).

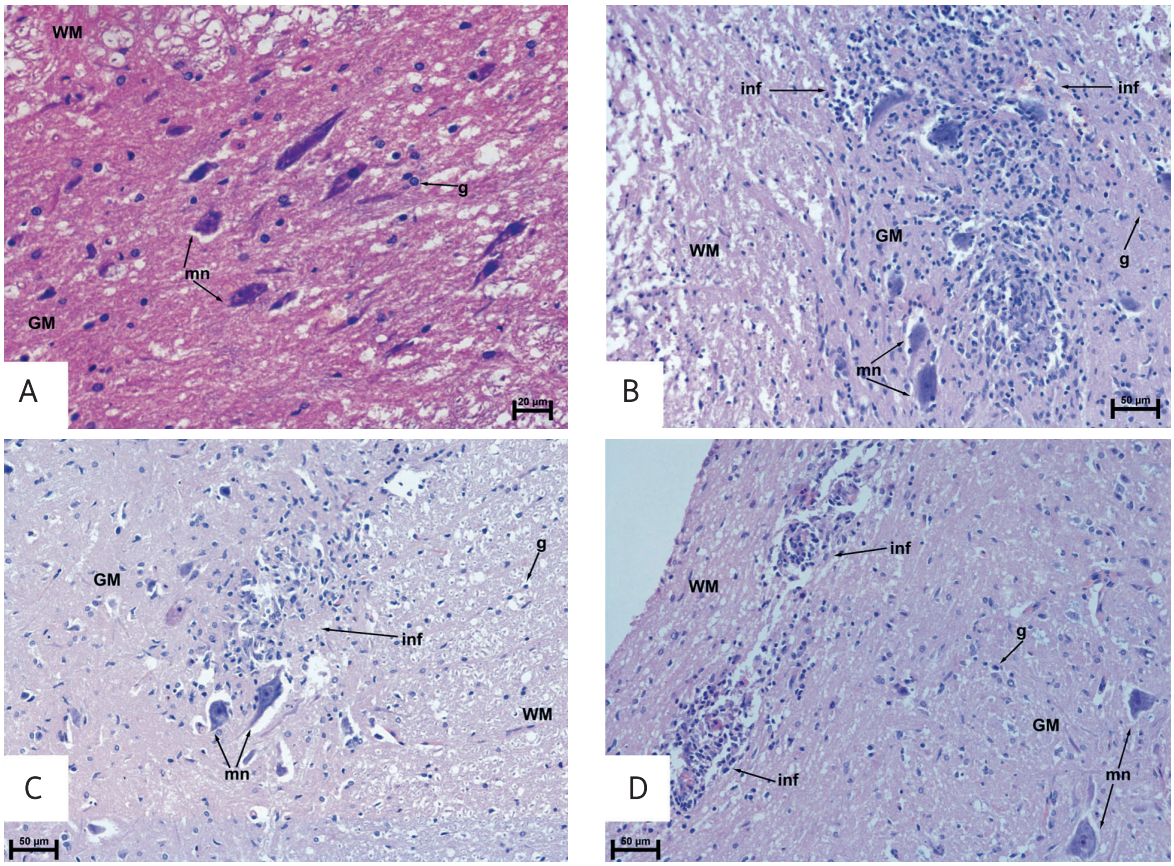

Fig. 7A-D. The microstructure of spinal cords of rats from the studied groups. The preparations were administered as described in Fig. 3. **A)** Cross-section of the spinal cord of a naive rat (normal appearance). Normal appearance of the white matter (WM) and grey matter (GM). In the grey matter glial cells (g) and motor neurons (mn) are visible, located in the ventral horn of the spinal cord, H&E. **B)** Cross-section through the spinal cord of a rat with fully developed EAE. In the grey matter (GM) extensive, inflammatory infiltration (inf), H&E. **C)** Cross-section through the spinal cord of a TFX® fraction II-treated rat. Inflammatory infiltration (inf) located around motor neurons (mn), H&E. **D)** Cross-section through the spinal cord of an IFN-β-treated rat. Inflammatory infiltrations (inf) concentrated around blood vessels in the borderline of white matter (WM) and grey matter (GM), H&E. Original magnification: A × 400; B, C, D × 200.

where about a two-fold increase of cytokine production was found in fraction II of TFX®- and IFN-β-treated rats ($P = 0.0034$ and $P = 0.0344$ for TFX® and IFN-β groups, respectively). Likewise, the production of IFN-γ was doubled in fraction II of TFX®- and IFN-β-treated rats ($P = 0.0201$ and $P = 0.0071$ for TFX® and IFN-β groups, respectively) (Fig. 6B).

Therapeutic effects of TFX® fraction II and IFN-β in histological, immunohistochemical and ultrastructural studies

Histological analysis revealed morphological differences in the microstructure of spinal cords of rats from the studied groups (Fig. 7). In the naive group (rats treated with 0.9% NaCl) the spinal cord presents a normal picture (Fig. 7A). In the control group with elicited EAE (no therapeutic treatment) numerous, extensive inflammatory infiltrations (inf) formed by lymphocytes, neutrophils and microglial cells were observed (Fig. 7B). The infiltrations were mainly located in the grey matter (GM) of the spinal cord, around blood vessels and perikarya of motor neurons (mn), as well as in the white matter (WM). The cytoplasm of motor neurons exhibited lucencies and cytoplasmic vacuolization, indicating degenerative processes. Proliferation of glial cells (g) was also registered. In fraction II of the TFX®-treated group (Fig. 7C) a reduction

of the infiltration area in the spinal cord was found. Inflammatory cells (lymphocytes, microglial cells) were present mainly around perikarya of the motor neurons. Neurodegenerative processes, such as vacuolization of the neuron cytoplasm, were rarely noted. The histological analysis of the spinal cords of the IFN-β-treated group (Fig. 7D) showed smaller inflammatory infiltrations in comparison to the control group. The infiltrations were present around the blood vessels, in the borderline of the grey and white matters. Cytoplasmic vacuolization in motor neurons was also observed.

The immunohistochemical examination revealed strong expression of the pro-inflammatory cytokines IL-6 and TNF-α within the spinal cord of rats not subjected to therapy (Fig. 8B). No staining for these cytokines was found in naive rats (Fig. 8A). Expression of IL-6 and TNF-α in rats from fraction II of TFX®- and IFN-β-treated groups was present but was less pronounced than in untreated rats (Fig. 8C and Fig. 8D).

The ultrastructural analysis of spinal cords from naive rats demonstrated normal features of myelin sheath, axoplasm and mitochondria (Fig. 9A). In contrast, in immunized rats, not subjected to therapy, extensive areas of myelin sheath delamination were found (Fig. 9B). In addition, distortions of the myelin sheath were observed. Furthermore, vacuolization of axoplasm and glial cell degeneration were noted. On the other hand, in fraction II of the TFX®-treated group (Fig. 9C), the areas of myelin sheath delamination were much smaller and focal, as compared to the rats given no therapy. In addition, axoplasm and glial cells exhibited no significant changes. In the IFN-β-treated group (Fig. 9D) delaminations of myelin sheath were seen as focal as well as more diffuse forms and were smaller as compared to rats with full manifestation of EAE.

Discussion

In this study we demonstrated that TFX®, applied daily from the day of immunization (preventive application), significantly inhibited the clinical signs of EAE. In subsequent experiments we demonstrated that Sephadex 25 fraction II of TFX®, administered therapeutically, significantly suppressed clinical symptoms, regulated immunological parameters, reduced histopathological changes, and normalized the ultrastructure of the spinal cord of Lewis rats with fully developed EAE. The efficacy of TFX® fraction II treatment, using the applied protocol, was even higher than that of IFN-β-1b, which is routinely used for amelioration of MS symptoms in patients.

For more than two decades no reports have appeared on the therapeutic effects of thymus hormones in the models of autoimmune encephalomyelitis. Kato and Nakamura [14] demonstrated that serum thymus factor was protective in the models of EAE in guinea pigs and rats. On the other hand, in a chicken model immunized with myelin basic protein (MBP) no effect of TFX® administration on the autoantibody levels was found [8].

The improvement of the clinical score by TFX® and its fraction II was well documented in this study and further confirmed by other parameters such as significant reduction of cell numbers in the draining lymph nodes and circulating blood leukocytes and regulatory effects of the preparation on cytokine levels in culture supernatants. In addition, the therapeutic efficacy of TFX® fraction II administration was supported by histological, cytohistochemical and ultrastructural analyses.

As could be expected, treatment with fraction II of TFX® significantly elevated concentrations of IL-10 induced by specific antigen in lymph node cell cultures (Fig. 6A). In addition, serum TGF-β levels were increased in this group of rats (not significantly, and therefore not shown), which could indirectly suggest activation of regulatory T cells [5]. An important role of IL-10 and TGF-β in inhibition of experimental encephalomyelitis was demonstrated by Rott *et al*. [24] and Wyss-Coray *et al*. [32], respectively. A possibility of induction of regulatory cells by TFX® awaits another study aimed at establishment of phenotypic changes of relevant cells during treatment of rats with TFX®. It will also be of interest to investigate whether TFX® inhibits IL-17 production – important in pathogenesis of EAE [19]. In addition, the stimulation of antigen-specific IFN-γ production by lymph node cells of rats treated with fraction II of TFX® (Fig. 6B) is consistent with the role of this cytokine in the control of EAE [1-3,20]. Likewise, as a result of an experimental therapy regimen involving oral myelin antigens together with IFN-β, an increase of IFN-γ production by lymphocyte culture was registered, accompanied by increased production of TGF-β, IL-4 and IL-10, the cytokines typical for Th2-type regulatory cells [21].

The improvement of the rat's mobility paralleled the histological and ultrastructural changes within

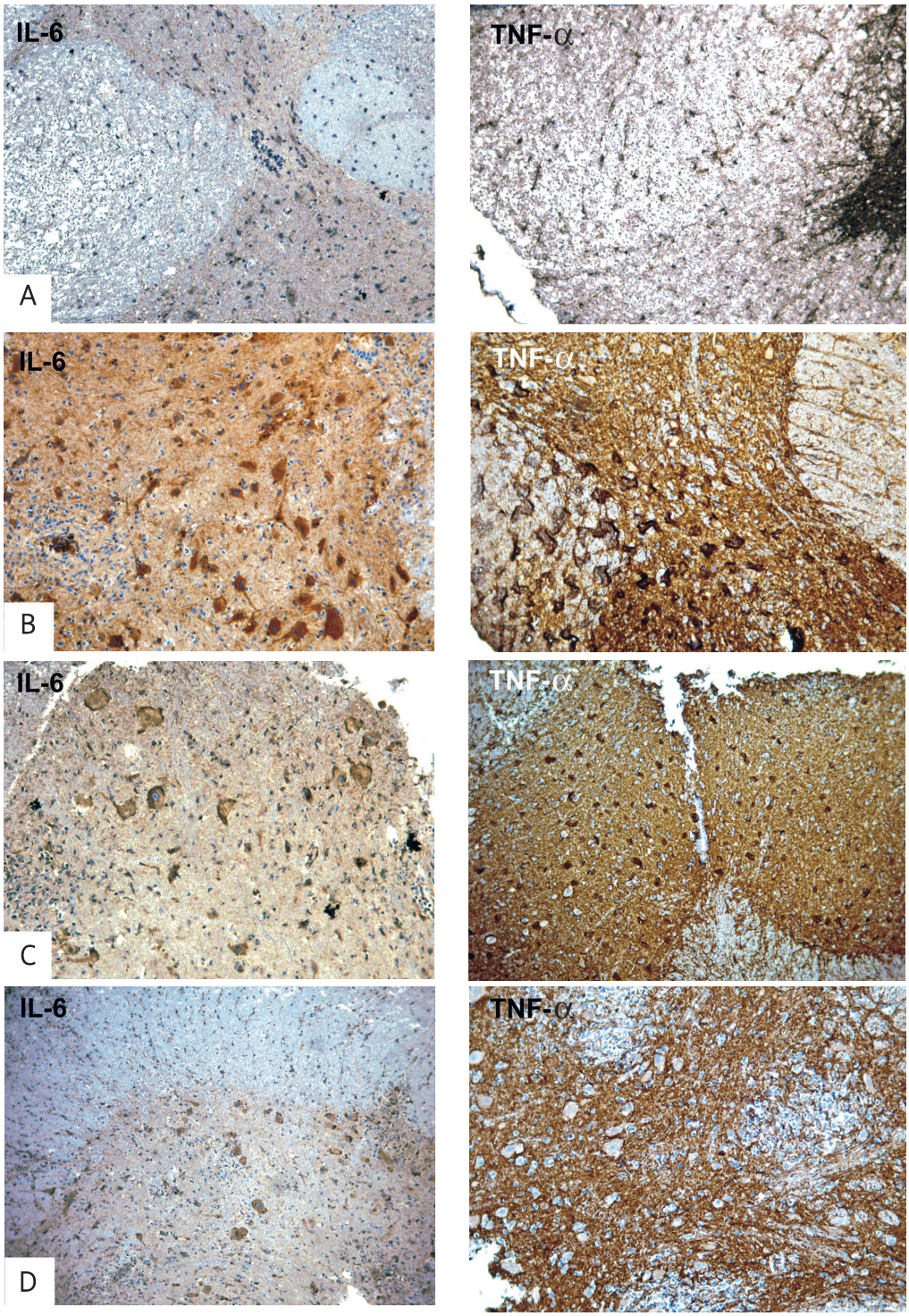

Fig. 8A-D. Immunostaining for the presence of IL-6 and TNF-α in the thoracic spinal cord. The preparations were administered as described in Fig. 3. **A)** The spinal cord from naive rats (no staining). **B)** The spinal cord of rats not subjected to therapy (strong immunostaining reaction within the examined tissue). **C)** The spinal cord of the TFX® fraction II-treated group (showing a moderate reaction, less pronounced as compared to the reaction in the appropriate control). **D)** The spinal cord of the IFN-β-treated animals (showing a moderate reaction, less pronounced as compared to the reaction in the appropriate control).

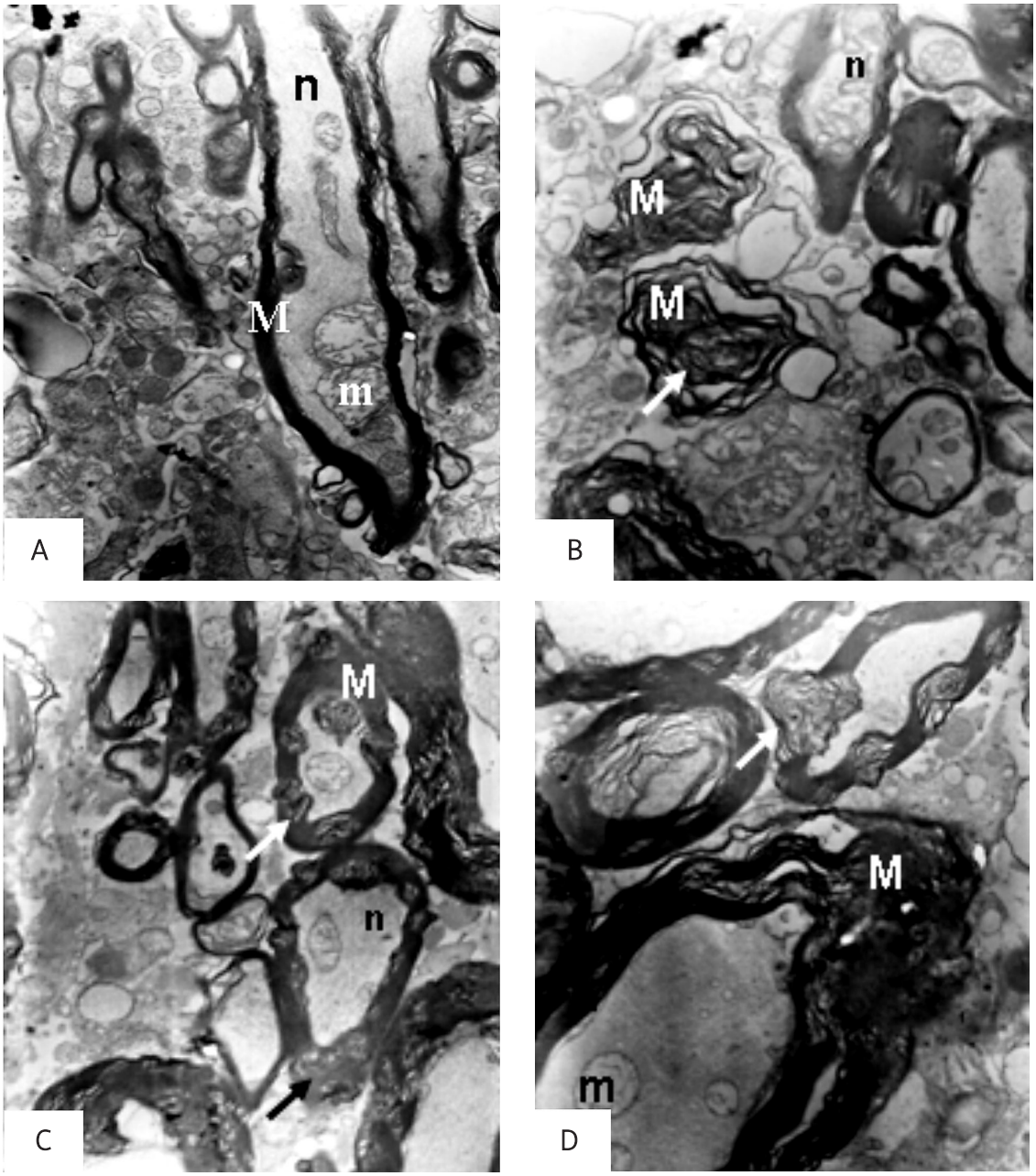

Fig. 9A-D. The ultrastructural analysis of spinal cords. The preparations were administered as described in Fig. 3. **A)** Electron micrograph of thoracic parts of a spinal cord section from naive rats, showing normal picture; myelin sheath (M), axoplasm (n), mitochondria (m) represent normal ultrastructural features. **B)** Electron micrograph of thoracic parts of a spinal cord section from rats with fully developed disease (no therapy); areas of myelin sheath (M) delaminations (white arrow); axoplasm (n) exhibits features of vacuolization and glial cell degeneration; some distortion of myelin sheath is also observed. **C)** Electron micrograph of a spinal cord section of the TFX® fraction II-treated group; focal delaminations of myelin (arrows) seen within myelin sheath (M) are much smaller as compared to rats with full manifestation of EAE. **D)** Electron micrographs of a spinal cord section of the IFN-β-treated group. Delaminations of the myelin sheath (M) are seen, in both focal (arrow) and more diffuse form, and are smaller as compared to rats with full manifestation of EAE. Original magnification: A, C × 24 000; B, D × 32 000.

the spinal cord. First, in TFX® fraction II-treated rats the infiltration of the spinal cord by neutrophils was strongly reduced (Fig. 7). This was also accompanied by reduction in the cellular expression of pro-inflammatory cytokines IL-6 and TNF-α (Fig. 8). All these changes clearly indicate suppression of the inflammatory process in the course of TFX® fraction II administration. Further, the treatment of EAE rats with TFX® fraction II led to amelioration of pathological changes in the electron microscopy analysis such as decrease in cellular vacuolization of glial cells and delamination of myelin sheaths (Fig. 9).

The good efficacy of IFN-β and VIP in amelioration of EAE symptoms was expected, taking into account their previously established activities [4,6]. We are, however, not certain whether the therapeutic protocols applying these preparations were optimal, so at this stage of investigation we cannot state which preparation ensures the best results in diminishing clinical and histological parameters of this experimentally-induced pathology. Nevertheless, the stability of TFX® fraction II found in our study (the preparation preserves its activity during storage at 4°C for months) gives the preparation an advantage over very unstable interferons.

Although the bulk of the TFX® activity is confined to fraction II, the fraction is still heterogeneous and may contain more than one active peptide which may act in synergy with another peptide (or peptides) to exert the therapeutic effect. Fraction I of TFX® may also contain an active peptide(s) since its action was significant as well. Therefore, it is essential to establish the minimal number of peptides in TFX® preparation responsible for its biological activity.

In summary, this study demonstrated the therapeutic utility of an extract from calf thymuses in amelioration of the symptoms of autoimmune encephalomyelitis in rats as evaluated by clinical, immunological, histological and ultrastructural parameters. Studies are underway to further characterize the active peptides present in the active fraction and to clarify their mode of action on the immune system.

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Conflict of interest

The authors state no conflict of interest.

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Polish patent description No. 108 714 (1981): A way of isolation of a preparation from calf thymuses stimulating immunity.