I. INTRODUCTION provided by Bionox

A. Thymus Gland

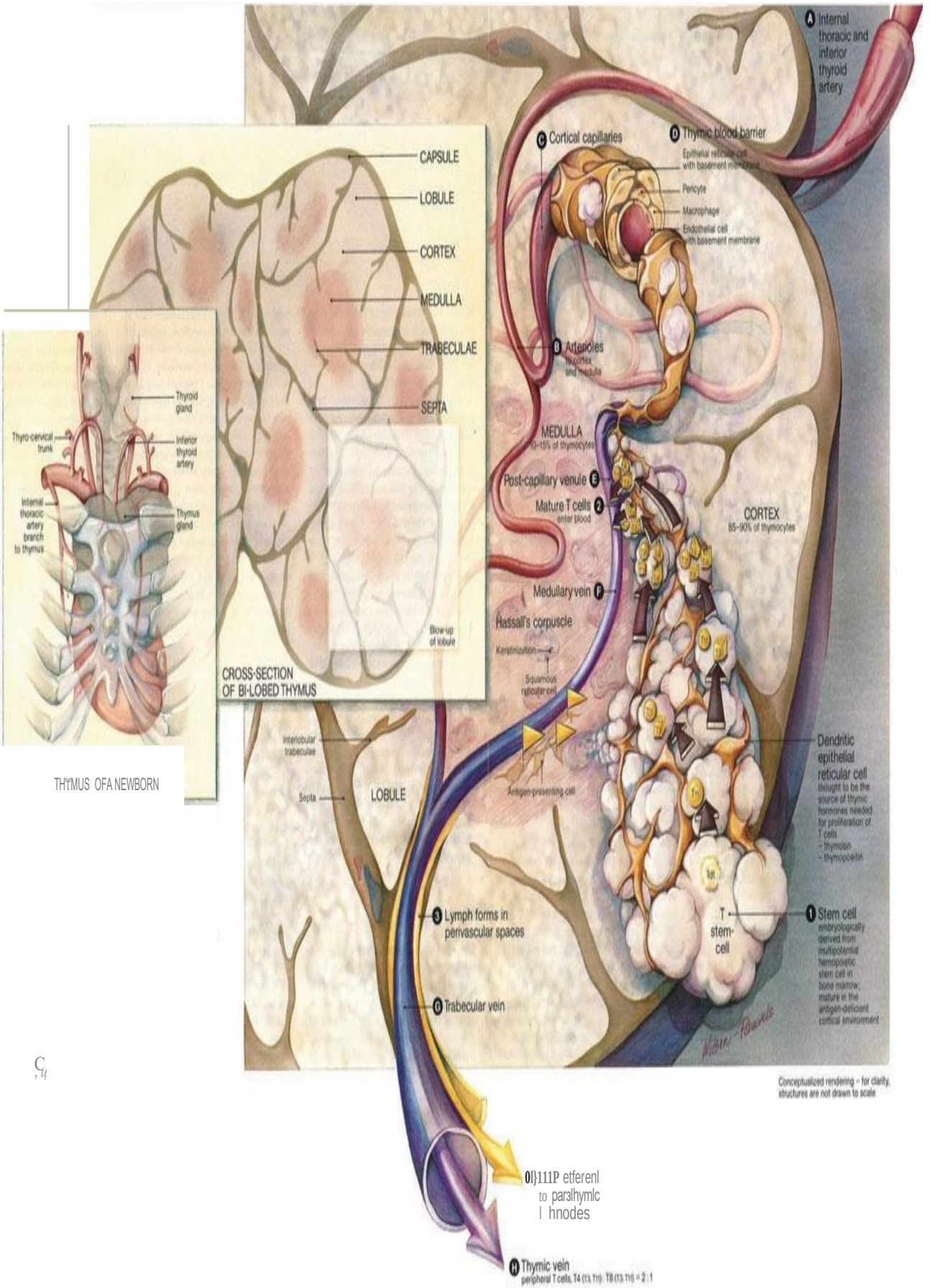
The thymus is a specialized organ of the immune system. The only known function of the thymus is the production and "education" of T-lymphocytes (T cells), which are critical cells of the adaptive immune system. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum (Figure 1, left).

Histologically, the thymus can be divided into a central medulla and a peripheral cortex which is surrounded by an outer capsule (Figure 1, middle). The cortex and medulla play different roles in the development of T-cells. Cells in the thymus can be divided into thymic stromal cells and cells of hematopoietic origin (derived from bone marrow resident hematopoietic stem cells). Developing T-cells are referred to as thymocytes and are of hematopoietic origin. Stromal cells include thymic cortical epithelial cells, thymic medullary epithelial cells, and dendritic cells (Figure 1, right).

The thymus provides an inductive environment for development of T-lymphocytes from hematopoietic precursor (progenitor) cells (Figure 2). In addition, thymic stromal cells allow for the selection of a functional and self-tolerant T-cell repertoire. Therefore, one of the most important roles of the thymus is the induction of central tolerance.

The thymus is largest and most active during the neonatal and pre-adolescent periods. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose (fat) tissue. Nevertheless, residual T lymphopoiesis continues throughout adult life

Figure 1. Thymus - anatomy, left, histology, middle, T-cell development, right (source: internet free domain.)


A Internal thoracic and inferior thyroid artery
C Cortical capillaries
D Thymic blood barrier
Epithelial reticular cell with basement membrane
Pericyte
Macrophage
Endothelial cell with basement membrane
B Arterioles
MEDULLA
10–15% of thymocytes
Post-capillary venule E
Mature T cells 2
enter blood
CORTEX
85–90% of thymocytes
Medullary vein F
Hassall's corpuscle
Keratinization
Squamous reticular cell
Interlobular trabeculae
Septa
LOBULE
Antigen-presenting cell
Dendritic epithelial reticular cell
thought to be the source of thymic hormones needed for proliferation of T cells
– thymulin
– thymopoietin
T stem-cell
1 Stem cell
embryologically derived from multipotential hemopoetic stem cell in bone marrow; mature in the antigen-deficient cortical environment
3 Lymph forms in perivascular spaces
G Trabecular vein
Conceptualized rendering – for clarity, structures are not drawn to scale
CAPSULE
LOBULE
CORTEX
MEDULLA
TRABECULAE
SEPTA
Blow-up of lobule
CROSS-SECTION OF BI-LOBED THYMUS
Thyroid gland
Thyro-cervical trunk
Inferior thyroid artery
Internal thoracic artery branch to thymus
Thymus gland
THYMUS OF A NEWBORN
0l}111P etferenl to parslhymlc l hnodes
H Thymic vein
peripheral T cells, T4 (T3, T1); T8 (T3, T1) = 2 : 1

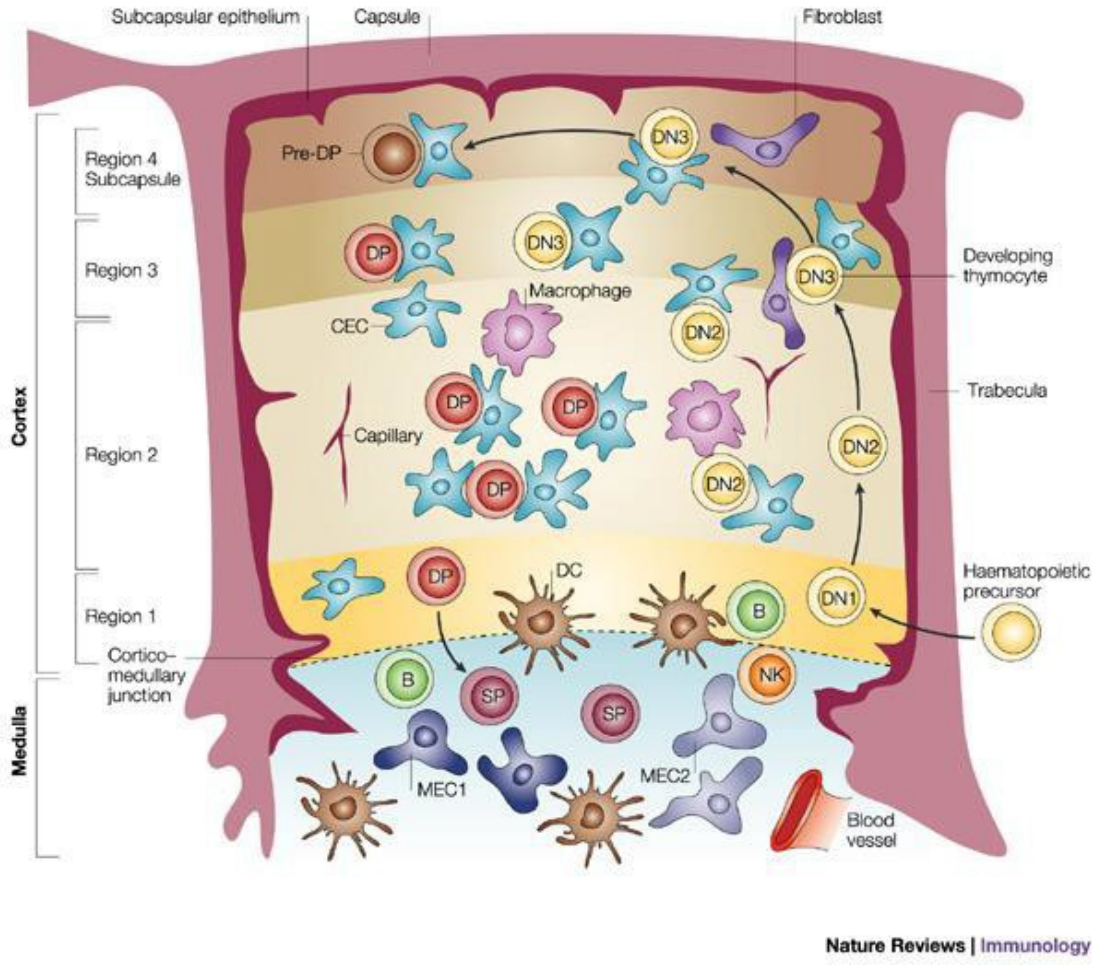


Figure 2. Development of T-lymphocytes in Thymus (source: Internet free domain - Nature Reviews)

In the past the thymus was viewed as more or less unnecessary. It was also believed to hinder the development of the sexual organs until puberty. It was not until 1905 that HAMMAR discovered new-born animals could not survive without a thymus gland. Studies done by KNIPPING (1924) came to the conclusion that the application of "fresh-pressed calves' thymus gland extract" lead to an increase in the lymphocytes and an increased resistance to infectious diseases. In 1962 MILLER discovered the true function of the thymus gland: a training centre for pre-t-lymphocytes.

B. Medical Application of Thymic Extracts

From 1953 until his death in 1989, SANDBERG regularly treated his patients with watery thymus extract (THX).

In 1975 thymus therapy using THX/THYMEX- L, introduced by PESIC, reached its peak in Germany.

Nowadays, different thymus extracts are available for the patient. For instance THYMEX-L, which is licensed as a remedy in Sweden under the name ENZYTHYM, is permitted in Germany and other European countries for clinical examinations. With the authorization of the Canadian drug authorities- Health Canada, some clinical examinations are being done with THYMEX-L and TFX-THYMOMODULIN in Canada. THYMEX-L has been approved for export, in small amounts, to the USA from Germany. A lot of thymus gland extracts like LTP, ProBoost, THYMU, THYM etc. exists on the food supplement market, as well.

C. Thymic Hormones

However, despite the effects of various of thymus gland extracts, the most promising therapeutic agents are represented by representatives of peptidic thymic hormones and especially by their short-chain analogues.

Singular thymus factors (peptides, hormones) influence various maturity levels in lymphocytes. Thymus peptides induce the expression of specific T-lymphocyte-receptors on immature precursor lymphocytes, as well as the differentiation of the T-lymphocytes in the thymus. During the therapeutic application of thymus preparations, the short-chained thymus peptides regulate the lymphocytes' sub-population. In addition the new formation of pre-thymocytes in the bone marrow is induced.

Up to now, more than twenty thymus peptides have been isolated from the whole-thymus extract. Five of them have already been investigated in depth.

- **Thymosin α_1** increases the interferon, the lymphokine, and the MIF (migration inhibitory factor) production. It also increases immunity against viruses, fungi, and tumors.

- **Thymosin β_4** stimulates the release of LH-RH (luteinising hormone - releasing hormone) and LH and induces the expression of receptors, in vitro and in vivo, in the bone marrow of healthy mice and in mice without a thymus, as well as in vivo in the thymocytes of mice with a depressed immune system.
- **Prothymosin α** is ten times more active than Thymosin α_1. Isolation in large quantities was not successful until some years ago in Germany. This break through made additional research possible. Prothymosin α has a strong immune regulating effect on the T-lymphocytes.
- **Thymic Humoral Factor (THF)** restores the splenocytes' in-vivo ability of new-born thymectomy donors to induce a graft vs. host reaction and intensifies the response of normal splenocytes to PHA- and Con-A-stimulation.
- **Thymopoietin** helps the intrathymic lymphocytes to differentiate amongst the bone marrow cells

D. Thymopoietin and its Analogues

Thymopoietin (abbr. Tpo, TP, approved gene symbol: TMPO) is a protein of 49 amino acids isolated originally from bovine thymic extracts and termed thymin.

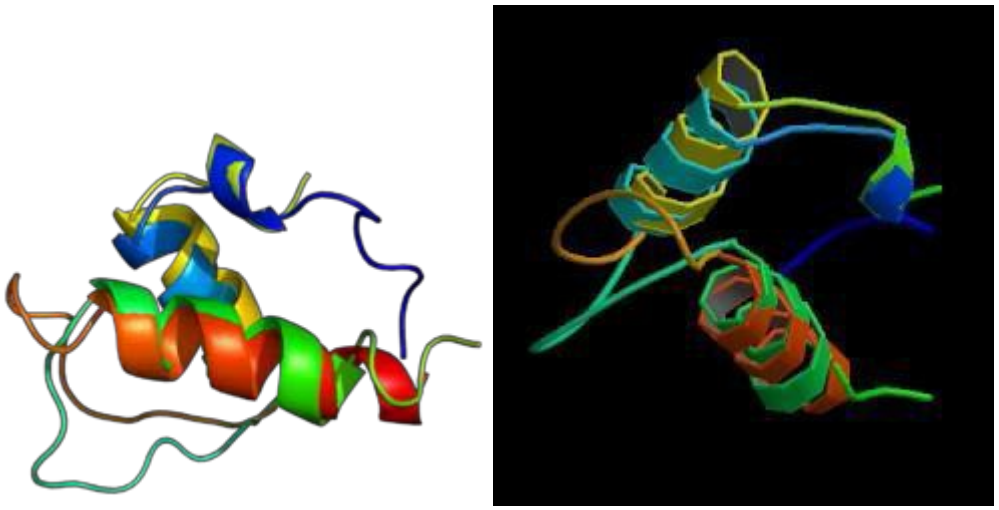


Figure 3. 3D structure of thymopoietin (source: Wikipedie)

Three human variants, termed TMPO-alpha (thymopoietin-alpha), TMPO-beta (thymopoietin-beta), and TMPO-gamma (thymopoietin- gamma),

having largely identical sequences have been described. TMPO- beta has been shown to be the human counterpart of rat LAP2 (Lamina- associated polypeptide 2).

Thymopoietin specifically interacts with the neuronal nicotinic Alpha-bungarotoxin receptor population and can regulate the toxin binding sites in chromaffin cells in culture. It can cause a complete block of contractile responses evoked by stimulation of the phrenic nerve diaphragm junction of the rat in vitro and is only slightly less potent than Alpha-bungarotoxin at concentrations of approximately 10^{-8}M. Thymopoietin induces the phenotypic differentiation of T precursor cells in vitro while inhibiting phenotypic differentiation of B-cells.

Thymopentin is able to increase IL2 production and IL2 receptor expression.The increased IL2 synthesis/IL2 receptor expression observed after treatment with thymopentin leads to an increased intrinsic T-cell responsiveness and probably also to an increase in the size of the responsive T-cell pool and thus may be the crucial mechanism of the immunopotentiating activity of the drug. TP-5 enhances bone marrow natural killer cells, probably by permitting the maturation of their precursors, and also natural killer activities in peripheral blood mononuclear cells of patients having received the drug three times per week for one month (50 mg sc). TP-5 has been found also to be useful in the treatment of a subgroup of patients with Sezary syndrome (acutaneous T-cell lymphoma). Thymopentin also induces ACTH-like immunoreactivity release by human lymphocytes.

Thymopoietin analogues

TP-5 (thymopentin or **thymopoietin 32-36**) is a synthetic pentapeptide (Arg-Lys-Asp-Val-Tyr) corresponding to amino acids 32-36 of thymopoietin. It appears to represent the active site of thymopoietin in that it has all the biological activities of the native hormone.

Some analogs of TP-5, for example TP-3 (Arg-Lys-Asp), and TP-4 (Arg-Lys-Asp-Val) exhibit significant immuno stimulating potencies in vitro and in vivo exceeding those of thymopentin.

E. Clinical Application of Thymopentin

The first generation of thymopoietin analogues is representad by thymopentin, known also as TH-5. The products containing TH-5 (TIMUNOX, Johnson&Johnson, MEPENTIL, Recordati, SINTOMODULINA,

Italfarmaco) are available on the market for about 20 years and are indicated as immunomodulators in primary immunodefficiences, secondary immunodefficiences, in complications of cancer patients after chemotherapy, and are also indicated for the stimulation of immune responses in conjunction.

II. NEW GENERATION OF THYMOPOIETIN ANALOGUES

A. Imunofan

IMUNOFAN, the new generation of small regulatory peptides derived from thymopoietin active center, has been developed by targeted modification of thymopoietin active center by the Russian group BIONOX in late 1980. Studying structure-activity relationships, and applying the AA sequence modifying approach it was determined that the exchage of AAs in position 2 and 3 of the 5 AA active center significantly enhanced the activity of a new peptide. In futher studies it was identified that the hexapeptide, containing Arg in position 6 reveals much higher activity than the original pentapeptide derived from thymopoietin active site. The new Arginyl-alpha-Aspartyl-Lyzyl-Valyl-Tyrozyl-Agrinine hexapaptide with the m.w. of 835.5 D was called Imunofan.

The peptide passed pre-clinical and clinical testing, including in vitro testing in tissues cultures of cell lines and human and animal lymphocytes, acute, subchronic and chronic toxicity testing in mice, rats and dogs, and clinical testing in patients.

B. Results of preclinical tests

Clinical testing showed the product is quite safe and efficient. Based on experimental data, the therapeutic dosage of 50 µg/ml per day (20 times less that in case of TH-5 analogue) has been determined. No toxic effect was identified even after the single dose of 1000 times of therapeutic dose in experimental animals. Due to short half life of the product (about 10 min.) no chronic toxicity, no signs of side effects were determined).

The trials with Imunofan have demonstrated that it is able restore:

- cell immunity,
- oxygen-dependent neutrophilic bactericidal system, and
- antiviral antibody production.

Imunofan also modulates both *in vitro* and *in vivo* levels of inflammatory mediators such as TNFα and IL-6, and activates the redox system.

Biological activity of Imunofan is documented by the following results of the preclinical and clinical trials (the trials were selected from Bionox file, all experiments and results were run by Bionox and are in full details described in Bionox files, all data belongs to Bionox).

B.1. The Effect of Imunofan on expression of HLA-DR, CD2, CD4,CD8, and CD22 on human peripheral mononuclear cells in vitro

PMN cells were separated on Ficol- Verografin gradient, washed and diluted by PRMI-1640 medium (5% of calf serum) to a final concentration of $2x10^6$ cell/ml and incubated in presence of 2.02, 0.1, 0.5, and 2.5 μg/ml of Imunofan for 1 h. RPMI-1640 was used in control. The expression of surface markers was determined by immune enzymatic, method using primary mouse mAbs to CD2, CD4, CD8, CD22, and HLA-DR, respectively. Cells were treated, fixed, washed, treated by peroxidase labelled conjugate and evaluated under 492 nm. The results, expressed in (%) of control, are summarized in Table 1.

Table 1. Expression of surface structures on human periferal PMN cells from healthy donors.

Imunofan µg/ml	HLA-DR	CD2	CD4	CD8	CD22
control	100.0±4.5	100.0±3.7	100.0±2.3	100.0±3.6	100.0±6.4
0.02	102.7±9.8	108.9±14.5	95.1±1.5	89.1±11.8	97.5±14.6
0.1	103.3±7.0	108.9±6.5	89.2±7.3	78.7±2.6	97.9±8.3
0.5	107.7±11.1	104.8±6.9	90.0±8.7	89.8±3.9	98.0±8.6
2.5	110.3±8.7	116.1±12.0	95.1±8.4	96.7±4.1	100.7±9.3

The results indicated the effect of Imunofan on the increase of HLA-DR and CD2 markers expression on the surface of PMN cells, i.e. the treatment by Imunofan is characterized by the increase of immune response (HLA-DR) and maturation of T lymphocytes (CD2).

B.2. The Effect of Imunofan on production of antibody producing cells in mouse spleen

Females of BCA/CaLaeSto strain (7 to 8 animals in a group) were immunized by $2x10^6$ sheep erythrocytes i.p. 10 to 15 min. later 0.5 ml of 0.02, 0.1, 0.5 and 2.5 µg /ml Imunofan was applied. 4 days later mice were sacrified and number of antidody producing cells was identified by using agar diffusion hemolysis of sheep erythrocytes.

Table 2. The effect of Imunofan on the quantity of antibody producing cells in mouse spleen

Imunofan, µg/ml	Number of Ab producing cells
control	100 (79±125)
0.02	371 (229±603)
0.1	192 (113±327)
0.5	171 (110±265)
2.5	228 (106±497)

The results indicate Imunofan influence on antibody producing cells characterized by minimum twofold increase after the treatment.

B.3. The effect of Imunofan on production of IL-2

The mice were treated by 0.05, 0.25, or 1.25 µg/mouse i.p. 1, 2, and 3 days later splenocytes were isolated and in vitro IL-2 production was determined.

$5x10^6$/ml mouse splenocytes or human PMN cells were cultivated in RPMI-1640 (2% calf serum) with 5µg/ml Con A for 20 to 24 hrs. IL-2 content was determined by IL-2 dependent CTLL-2 cell line bioassay. The assay was calibrated by recombinant IL-2 (0.5 to 50.0 IU/ml).

Table 3. The effect of Imunofan in IL-2 synthesis.

Imunofan µg/mouse	Days after 1	Imunofan 2	Treatment 3
control	21.6±1.6	21.6±1.6	21.6±1.6
0.05	50.4±6.6	48.7±5.5	35.5±3.9
0.25	21.5±3.3	67.2±13.2	13.9±1.3
1.25	24.6±8.1	46.9±3.75	157.4±13.5

As evident from the results any of tested dosage of Imunofan induced synthesis of IL-2, however, the dose dependent induction had a different time response. While 0.05 µg induced the highest peak of IL-2 synthesis after 24 hrs, 0.25 µg induced the highest peak after 48 hrs and 1.25 µg induced the peak after 72 hr., respectively.

B.4. The effect of Imunofan on IL-2 synthesis of Cyclosporine A suppressed cells

Two strains of mice, DBA/2 and Balb/c, were treated by Cyclosporine A (100µg/kg) , followed by 0.05 µg Imunofan/animal i.p. Splenocytes were cultivated for 24 and 48 hrs *in vitro* with ^{3}H Thymidine with or without presence of recombinant IL-2 (5 IU/ml).

Table 4. The effect of Imunofan on IL-2 synthesis in cyclosporin A treated cells.

Mouse strain	Group No.	Treatment	^{3}H Thymidine Incorporation After 8 hrs	After 48 hrs
DBA/2	1	Ery	10960±2517	13040±478
DBA/2	2	Ery+CyA	9720±719	2079±33
DBA/2	3	Ery+CyA+IL-2	7571±322	10351±367
Balb/c	1	Ery	25596±2249	20381±212
Balb/c	2	Ery+CyA	9578±1676	11315±887
Balb/c	3	Ery+CyA+IL-2	12288±1278	23268±298

The experiments with Cycloporine A showed that Imunofan significantly diminished the effect of Cyc A and restored sensitivity of mouse splenocytes to recombinant IL-2. The effect was no strain dependent.

B.5. Production of IL-2 in splenocytes from irradiated animals

CBA strain males were treated by i.p. Imunofam, followed by irradiation with Cs^{137} (200 Rad). 4 days after irradiation splenocytes were isolated, diluted ($5x10^{6}$/ml), stimulated by 10 μg/ml Con A and tested for IL-2 production by using CTLL-2 bioassay.

Table 5 The influence of Imunofan on IL-2 production of splenocytes from irradiated mice (expressed as incorporation of 3H Thymidine into DNA of CTLL-2 cells cultivated with supernatant of cultivated splenocytes).

control	Irradiated mice	Concentration of Imunofan 0.05 μg/ml	0.25μg/ml
32781±1768	18899±1501	29982±1916	25575±1297

As evident from results, the treatment of irradiated mice with Imunofan resulted in increased, or even restoration, of production of IL-2.

B.6. The effect of Imunofan on TNFα production in mice after PLS induction

Mice of Balb/c and DBA/2 strains received 5 – 7 μg LPS i.p. followed by i.p. injection of Imunofan. 2 hours later the animals were sacrified and TNFα in serum was analyzed by TNF-sensitive cell line L-929. The results are summarized in Tab. 6.

Table 6. The effect of Imunofan on production of TNFα in LPS treated mice.

Imunofan μg/mouse	Bal Dilution 1:4	b/c of serum 1:8	DB Dilution 1:4	A/2 of serum 1:8
control (no imunofan)	0.538±0.01	0.456±0.07	0.07±0.03	0.06±0.007
0.01	0.651±0.04	0.486±0.04	0.138±0.02	0.181±0.015
0.05	0.818±0.04	0.773±0.05	0.130±0.006	0.147±0.025
0.25	0.891±0.06	0.757±0.05	0.071±0.021	0.043±0.008
Normal serum	0.713±0.008	-	0.211±0.032	0.413±0.061

As evident from the Table 6, co-administration of Imunofan with LPS inhibits in vivo production of TNFα (as higher optical density, as higher TNFα inhibition).

B.7. The effect of Imunofan on TNFα production in vitro

In another experiment, the effect of Imunofan on production of TNFα has been tested *in vitro*. It has to be stressed that the effect of Imunofan on TNFα synthesis by mononuclear cells from healthy volunteers was dependent on TNF level produced by tested cells. Synthesis of cells isolated from healthy volunteers varied in a broad interval from 100 to 1,500 μg /ml. The results clearly indicated the presence of volunteer with naturally high levels of TNFα, and opposite those with low levels of TNFα. To test the effect of Imunofan on TNFα production *in vitro*, two sets of donors, with less than 300 μg/ml and with more than 300 μg/ml in tissue culture medium, respectively. The results are summarized in Table 7.

Table 7. The effect of Imunofan on spontaneous production of TNFα by human mononuclear cells (results are expressed as % of the corresponding control)

Imunofan, μg/ml	Level of spontaneous by human mononuclear less than 300	production of TNFα cells more than300
control	100±3.2	100±8.3
0.01	152±10.3	-
0.05	150±12.1	80.4±12.4
0.25	133±5.4	-
1.25	195±30.3	74.6±4.0

The results showed that Imunofan increased TNFα production in cells from donors with levels below 300, however, decreased TNFα production in cells from high producing group. It seems that Imunofan has the ability to modulate TNFα production, i.e. stabilize its production on a level which could have physiological effect.

B. 8. Antioxidant effect of Imunofan

Two different mechanisms of Imunofan antioxidant activity were demonstrated *in vitro*. Imunofan was found to inhibit formation of free oxygen radicals , and second, Imunofan was found to increase synthesis of SOD in dose-dependent manner. In concentration of 1μ/ml Imunofan increased synthesis of SOD two times after 16 hrs. incubation, however, in concentration of 10μg/ml the synthesis increases 10 times.

C. Imunofan in Cancer

Included into the complex therapy of patients with cancer diseases, Imunofan enhances the bodys reserve capacity to inactivate free radicals and oxidants, substantially shortens radiation and toxic reactions. Its use ensures the continuum of chemoradiotherapy.

D. Imunofan in treatment of infectious diseases

Used in the complex therapy of chronic infections, Imunofan enhances antiviral and antibacterial immunity, shortens the manifestation of clinical symptoms and major syndromes of diseases.

D.1. Impact of Imunofan on production of virus-neutralizing antibodies upon immunization with infectious virus rhinotracheitis - infectious pustular vulvovaginitis vaccine (IRH-IPV)

During the works on development of inactevated vaccine for IRH-IPV for the cattle (done by Sverdlovskiy Inst.) experimental series was developed, however it did not show immunizing power high enough so that further work has been conducted to improve vaccine immunogenic activity by addition of immunomodulatory medication Imunofan [33] to vaccination protocol.

Use of single vaccine induces production of virus-neutralizing antibodies in 1:4 - 1:16 titer after 30 days since immunization. However, by day 90 since immunization specific antibody titer drops down to 0 - 1 :4. One-time injection of Imunofan the day before vaccination in the dose of 1 μg/kg per body weight induces increase of neutralization reaction indicant at the presence of virus IRH-IPV antibodies up to level of 1:32-1:128 after 30 days. Also high levels of protective antibody titers were observed over the whole trial period.

Thereby addition of Imunofan to immunization protocol significantly increase level and term of virus-neutralizing antibody circulation.

D.2. Impact of Imunofan on immunogenicity of tick-borne encephalitis vaccine

Trial was conducted with the use of Balb/c mice with 12-14 g body weight. Branch standard normal (BSN) of tick-borne encephalitis vaccine (cultural, inactivated, fiber-entrapped, fluidus) VKE 201 series developed by Institute of polyomyelitis and encephalitis of Russian Academy of Medical Science (RAMI) of Moscow was used for vaccination

VKE dilutions of 1:10, 1:32, 1:100, 1:320 were subdermally injected in the volume of 0,5 ml. Imunofan was subdermally injected in the dose of 0,05 μg per one mouse in the volume of 0,3 ml. Vaccine and Immunofan were injected both at once three times with one day stretch. Each dilution was injected to 10-12 mice. Tick-borne encephalitis of Absettarov strain was injected i.p. in the volume of 0,25 ml on the Day 9 after the end of immunization. Inoculating dose of tick-borne encephalitis virus was 320 LD_{50}. Mice were observed for 14 days after tick-borne encephalitis virus inoculation.

Table 8. Impact of Imunofan on immunogenicity of tick-borne encephalitis vaccine

Animal group	Vaccine dilution	% of survived animals	PR_{50}	MID_{50}
Control group (VKE)	1:10	80	1:38	0.013
	1:32	60		
	1:100	20		
	1:320	0		
VKE + Imunofan	1:10	100	1:72	0.007
	1:32	63.6		
	1:100	50		
	1:320	0		

As the Table 8 shows, with 1:10 vaccine dilution all mice received both vaccine and Imunofan have survived, at the same time among mice received vaccine only survival rate was 80%. In the dilution of 1:100 vaccine protected 20% mice, vaccine together with Imunofan showed to be 2.5-fold more effective and protected 50% animals. With vaccine dilution of 1:32 and 1:320 impact of Imunofan on VKE immunogenicity was not revealed. PR_{50} value increased 2-fold, MID_{50} value decreased 2-fold , i.e. to protect 50% animals in case of combination of vaccine and Imunofan it is possible to use 2-fold more diluted vaccine (value PR_{50}) or of the same vaccine concentration but 2-fold lower dose (value MID_{50}) compared to the group without Imunofan [15,28]. Thereby addition of Imunofan to tick-borne encephalitis vaccine immunization protocol allows significantly increase its immunogenicity and animal survival rate after virus inoculation.

D.3. Experimental study of Imunofan impact on immunogenic activity of vaccine against hepatitis A

Trial was conducted with the use of out-bred cavy with 300-350 g body weight. Each group included 10 cavy [28]. Experimental vaccine against hepatitis A (cultural, concentrated, purified, inactivated, fluidus) - Hep-A-in-VAK, series 16 (produced by Research and Development company NPO "Vector" of Novosibirsk) was used for the trial. Vaccine was subdermally injected into 3 spots of an animal in the area of rear pads and back with total volume of 0.5 ml. Imunofan in the dose of 1.5 mkg per one cavy was subdermally injected in the volume of 0.5 ml into cavy's back area. Vaccine and Imunofan were injected together, 3 times with two-week period. Immune strength against hepatitis A was evaluated by specific serum antibody titer (AT) towards hepatitis A virus (VHA) and by seropositive animal number. Heart blood sampling was done twice in immunization time course on the Day 14 after second and third injections. Anti-VHA-antibody titer was estimated in serum samples by diagnosticum "IFA-anti VHA" (developed by Research and Development company "Diagnostic systems" of Nijniy Novgorod).

Table 9. Impact of Imunofan on immunogenic activity of hepatitis A vaccine.

Animal group	Period of blood sampling	Geometric mean AT titer	AT titer	Rate of seropositive animals %
HepA-in-VAK + Imunofan	I	1.93±0.30	1:85	90
	II	2.97±0.25	1:933	100
HepA-in-VAK (control)	I	1 .40±0.34	1:25	70
	II	2.24±0.39	1:174	90

As the Table 9 displays, after two vaccine injections (1-st period of sampling) group of cavy received vaccine together with Imunofan (trial group) showed AT titers 3,4-fold higher than control group (1:85, 1:25 accordingly). After 3-time injections of medications AT level in the group of Imunofan was found 5,36-fold higher than in the group of vaccine only (1:933. 1:174, accordingly).

Rate of seropositive animals (AT titer higher 1:10) in control group was estimated 70% (1^{st} period of sampling) and 90% (2^{nd} period of sampling), and in trial group it was estimated 90% (1^{st} period of sampling) and 100% (2^{nd} period of sampling).

Thus, trial with cavy demonstrated stimulating effect of Imunofan on immunogenicity of hepatitis A vaccine.

The results of experimental study of peptide immuno-regulatory drug Imunofan have demonstrated ability of the drug to improve immune response upon injection of vaccine with reduced antigen content, improve protective quality of vaccine and increase animal survival rate after virus inoculation.

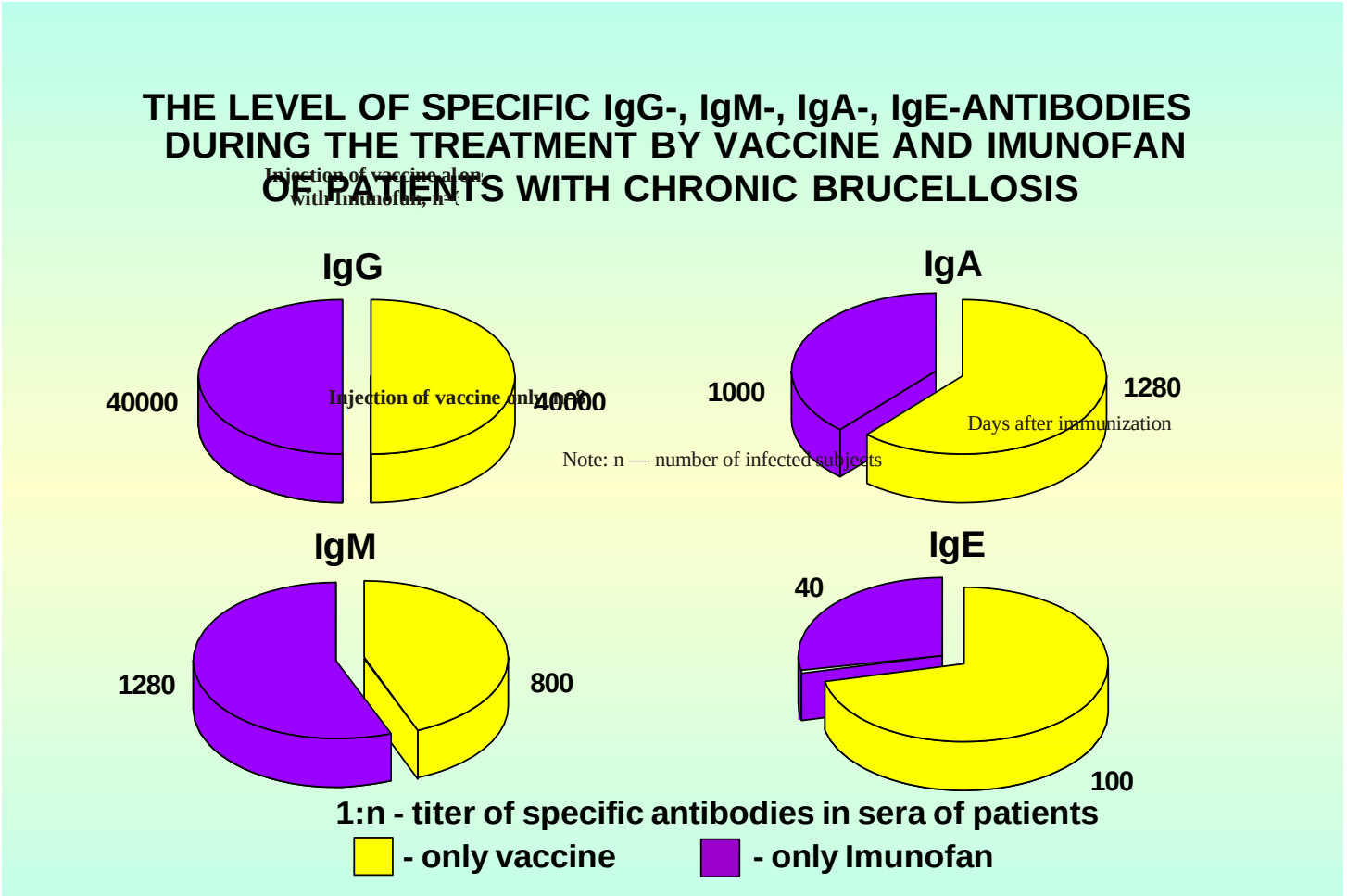
D.4. Imunofan in brucellosis patients

To evaluate the efficiency of peptide immuno-regulatory drug use compared to therapeutic vaccine activity, clinical-immunologic study of Imunofan administration in patients with brucellosis was conducted. This pathology was picked out due to the fact that brucellosis therapeutic vaccine has broad use to induce specific antibodies for treatment of the disease. Along with that, administration of therapeutic brucellosis vaccine causes a number of side-effects related to reactogenecity of vaccine and increase of allergisation among patients. For example, an increase of specific antibody titres among main classes of immunoglobulins IgM, IgG and IgA after vaccine injection also induces significant increase of IgE class antibody titres. Administration of Imunofan by brucellosis patients provides stimulation of specific antibodies of all main classes IgM, IgG, IgA approximately at the same levels as with therapeutic brucellosis vaccine injection. However the

level of reactive IgE class antibody production upon administration of Imunofan was almost 2.5-fold lower compared to the effect of therapeutic brucellosis vaccine injection (Figure 4). At the same time Imunofan administration induced decrease of IgE serum levels in patients with initially high levels of serum IgE.

Administration of therapeutic immuno-correcting agent by patients with brucellosis provides certain advantages compared to therapeutic vaccine activity and allows to prevent side-effects, increase of IgE class reactive antibody level and further allergisation of an organism.

Figure 4. Comparison of vaccination and Imunofan treatment on IgG, IgM, IgA and IgE levels in brucellosis patients



D. 5. Clinical- immunological effects of Imunofan treatment in chronic active hepatitis B (CAHB) patients

102 patients suffering from chronic hepatitis B were treated by imunofan for three weeks by 8 to 10 injections. In case of recurrence the treatment was repeated in 4 to 6 months. The patients were followed at day 0 (control), day 3, day 7 and day 28, respectively.

The results of immunological tests revealed partial lymphopoenia with a tendency to decrease of absolute number of B-lymphocytes (CD22), T-helpers/inductors (CD4) T-suppressors/cytotoxic cells (CD8) and activity of natural killers (NK) with corresponding increase matured T-lymphocytes (CD2).

Significant increase of CD4 and CD8 T-lymphocytes and NK cells was determined immediately (3 days) after the treatment by Imunofan. Positive effect of Imunofan characterized by regulation of T-lymphocytes subpopulations was evident during the whole period of treatment.

The results of Imunofan treatment on markers of humoral immunity are summarized on Figure 1. The decrease of HBe antigen was detected 3 days after Imunofan injection, it disappeared in all patients after 7 days followed by appearance of anti-HBe antibodies in almost all patients after 28 days. Elimination of HBe antigen together with appearance of anti-HBe antibodies showed the effect (targeting of) of Imunofan treatment on the replication of hepatitis B virus. The first evidence of the decrease of synthesis of HBs antigen appeared 7 days after the beginning of Imunofan therapy. After 28 days the elimination of virus antigen was evident in almost 50% of patients, accompanied by synthesis anti-HBs antibodies in almost 20% patients.

Imunofan treatment increased adhesion capacity and oxygen-dependent bactericidal ability of neutrophils, respectively, as markers of completed phagocytosis.

The stimulation of antiviral immunity and targeting of virus replication was manifested by improvement of biochemical markers of the activity of the pathological process. The activity of aminotransferases was decreased just 3 days after Imunofan application, after 28 days the activity of AST decreased from 401±182 to 242±77 (by about 60%) and the activity of ALT decreased from 747±293 to 314±84 (by about 42%). The decrease of the activity of serum aminotransferases was accompanied by 1.8 – 3 time decrease of bilirubin, as well.

Clinical effects were characterized by the decrease of complaints of astheno-vegetative and dispeptic disorders, decrease of the size of both liver and spleen and deletion of hemorrhagy.

Figure 5.

The effect of Imunofan treatment on markers of humoral immunity in hepatitis B patients