Effectiveness of long-term continuous immunomodulatory therapy with gamma-recombinant interferon in patients with clinically manifest forms of neutrophil myeloperoxidase deficiency

Efectividad de la terapia inmunomoduladora continua a largo plazo con interferón gamma recombinante en pacientes con formas clínicamente manifiestas de deficiencia de mieloperoxidasa de neutrófilos

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Abstract

Objectives: To date, the medication of gamma-recombinant interferon is employed to prevent infectious episodes while treating some phagocytic immunodeficiencies, including chronic granulomatous disease, autosomal dominant osteopetrosis of Type I, and defects of IL-12/IL23/gamma-interferon path. The objective of the study was to evaluate the efficacy and safety of long-term continuous immunotherapy with gamma-recombinant interferon in another phagocytic immunodeficiency, namely, a clinically manifested neutrophil myeloperoxidase deficiency.

Materials and methods: The experience in applying gamma-interferon in a dose of 500 thousand - 1 million IU per day overnight for 5-7 consecutive months was analyzed in retrospect. The diagnosis was confirmed based on a series of quantitative (flow laser cytofluorimetry) and qualitative (cytochemical method) measurements except for secondary immunosuppression causes. The control group consisted of 32 patients with myeloperoxidase deficiency, who refused the therapy, and the study group comprised 47 similar patients, who had undergone complete immunotherapy. The obtained data were analyzed following the parametric Student's t-test calculating the coefficient of significant difference p, and the number of Z-signs according to Urbach non-parametric test.

Results: The activity of neutrophil myeloperoxidase increased significantly in 45 out of 47 patients in the study group and only in 12 out of 32 control patients ($p<0.05$; $Z<0.05$). The content of myeloperoxidase in neutrophils increased significantly in 41 out of 47 patients of the treated group and only in 11 out of 32 control group patients ($p<0.05$; $Z<0.05$). In the treated group, the number of infectious episodes was reduced by 75%, and that of allergic episodes - by 51% ($p<0.05$; $Z<0.05$). A dramatic reduction in the necessity to apply antimicrobial therapy, readmission to hospitals, and surgical interventions ($p<0.05$; $Z<0.05$) has been established.

Conclusions: Application of recombinant human gamma-interferon can be an effective and safe preventive therapy strategy for myeloperoxidase deficiency phagocytes. Further research efforts with more participants and better designs are required.

Keywords: myeloperoxidase deficiency, interferon-gamma, immunotherapy.

Resumen

Objetivos: Hasta la fecha, la terapia con interferón gamma recombinante se emplea para prevenir episodios infecciosos en el tratamiento de algunas inmunodeficiencias fagocíticas, incluida la enfermedad granulomatosa crónica, la osteopetrosis autosómica dominante de tipo I y los defectos de la vía de IL-12 / IL23 / interferón gamma. El objetivo del estudio fue evaluar la eficacia y seguridad de la inmunoterapia continua a largo plazo con interferón gamma recombinante en otra inmunodeficiencia fagocítica, a saber, la deficiencia de la mieloperoxidasa del neutrófilo manifestada clínicamente.

Materiales y métodos: Se analizó retrospectivamente la experiencia en la aplicación de gamma-interferón en una dosis de 500 mil - 1 millón de UI diarias durante la noche durante 5-7 meses consecutivos. El diagnóstico se confirmó en base a una serie de medidas cuantitativas (citofluorimetría láser de

flujo) y cualitativas (método citoquímico) excepto por causas secundarias de inmunosupresión. El grupo de control estuvo formado por 32 pacientes con deficiencia de mieloperoxidasa, que rechazaron la terapia, y el grupo de estudio estuvo formado por 47 pacientes similares, que se habían sometido a la inmunoterapia completa. Los datos obtenidos se procesaron mediante la prueba paramétrica de t de Student con el cálculo del coeficiente de diferencia significativa p, y el número de signos Z según la prueba no paramétrica de Urbach.

Resultados: La actividad de la mieloperoxidasa de neutrófilos aumentó significativamente en 45 de 47 pacientes en el grupo de estudio y solo en 12 de 32 pacientes de control (p<0,05; Z<0,05). El contenido de mieloperoxidasa en los neutrófilos aumentó significativamente en 41 de los 47 pacientes del grupo tratado y solo en 11 de los 32 pacientes del grupo de control (p<0,05; Z<0,05). En el grupo tratado, el número de episodios infecciosos se redujo en un 75% y el de episodios alérgicos en un 51% (p<0,05; Z<0,05). Se estableció una reducción drástica en la necesidad de aplicar compuestos antimicrobianos, reingreso a hospitales e intervenciones quirúrgicas (p<0,05; Z<0,05).

Conclusiones: La aplicación de interferón gamma humano recombinante puede ser una estrategia de terapia preventiva eficaz y segura para la deficiencia de mieloperoxidasa de los fagocitos. Se requieren más esfuerzos de investigación con más participantes y un mejor diseño.

Palabras clave: deficiencia de mieloperoxidasa, interferón gamma, inmunoterapia.

Introduction

Human gamma-recombinant interferon drugs are still widely used in clinical practice to prevent infectious episodes of chronic granulomatous disease[1]. This immunotherapeutic agent has also demonstrated efficacy in other immunodeficiency disorders associated with phagocytic dysfunction, namely, congenital osteopetrosis of Type I[2], IL-12/IL-23 pathway defects[3,4], and selective gamma-interferon deficiency[5]. Accordingly, high-dose therapy with gamma-recombinant interferon has recently been reported been to be crucial in eliminating the manifestations of osteomyelitis caused by the vaccine strain of mycobacteria in a child with partial autosomal-dominant deficits of Type I receptors in gamma-interferon[6].

Neutrophils are no longer seen as leukocytes with the sole function of being the essential first responders in the removal of pathogens at sites of infection[7]. Being armed with numerous pro- and anti-inflammatory mediators, these phagocytes can also contribute to the development of various autoimmune diseases and can positively or negatively regulate the generation of adaptive immune responses[8]. Myeloperoxidase, the most abundant neutrophil granule protein, plays a key role in the various functions of neutrophils in innate and adaptive immunity.

Neutrophilic myeloperoxidase is characterized by direct bactericidal, fungicidal, and virucidal properties that determine its role in antimicrobial immunity, which constitutes the basis of human health[9]. Besides, myeloperoxidase can have also an immunoregulatory effect, which is important in maintaining immune tolerance and preventing autoimmune and allergic complications[10]. Neutrophil MPO suppresses DC function and adaptive immunity. Bell et al.[11] showed that rapidly infiltrating neutrophils release MPO in draining lymph nodes (LN) after antigen/adjuvant injection.The deposited MPO suppresses various aspects of DC functionincluding costimulatory molecule (e.g., CD86) expression and cytokine (IL-12, IL-23) production and migration, resulting in decreasedgeneration of CD4 T cell responses including T cell activation (CD44 expression), proliferation, and differentiation intoTh1 (IFN-producing) and Th17 (IL-17A-releasing) effectors.

Neutrophils myeloperoxidase-deficiency is a widespread immunodeficiency disease caused by the disruptions in the processes of chlorine-dependant oxidation of pathogens in neutrophils and monocytes/macrophages[12]. In the countries of Western Europe and the USA, such immunodeficiency occurs with the frequency of 1 case per 2000-4000 inhabitants[13]. It has been established that in 50% of cases, myeloperoxidase deficiency is asymptomatic, although in half of the patients, clinical manifestations of the disease were observed, and in 10% of persons, these symptoms became severe and even life-threatening. Immunodeficiency is manifested as a recurrent bacterial and fungal infection caused by opportunistic microflora and some non-infectious lesions[14]. Among bacterial pathogens, resistance to streptococcus has been particularly reduced[15]. Patients lacking MPO are more susceptible to fungal infections, particularly those, caused by Candida albicans. Severe, invasive candidiasis is considered the hallmark of this immune dysfunction[16]. However, particular cases have been reported, such as subacute infectious endocarditis caused by Prototheca wickerhamii[17]. Allergic and autoimmune complications have been reported in myeloperoxidase deficiency, including Ramsaransing[18] multiple sclerosis, Bell[11] rheumatoid arthritis, and Patıroğlu[19] chronic rheumatic fever. In some patients with myeloperoxidase deficiency, oncological manifestations such as myeloproliferative disease may develop.

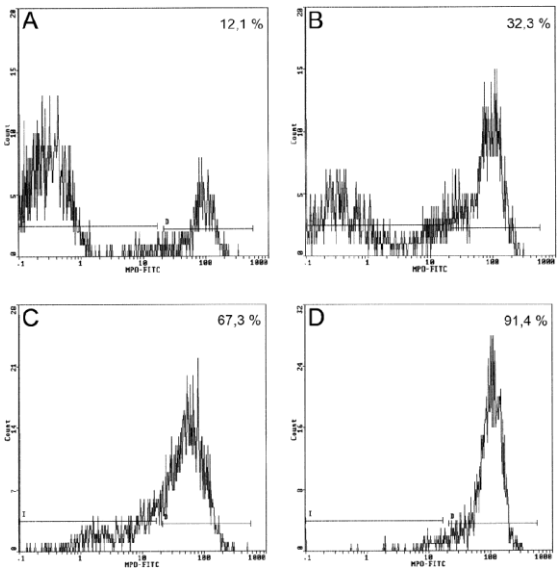
Nowadays, infectious manifestations in myeloperoxidase (MPO) deficiency are treated with antimicrobial chemotherapy drugs, although in the case of this immune dysfunction, the processes of drug biotransformation are violated, which results in poor tolerance of chemotherapy[20]. An alternative to this might be preventive immunotherapy in the same way as in chronic granulomatous disease. The reported clinical cases demonstrate the obvious benefits of gamma-recombinant interferon for the prevention and mitigation of infectious episodes in patients with clinically diagnosed myeloperoxidase deficiency[21-23]. The results of a recent uncontrolled trial demonstrated a significant clinical improvement in patients with this immunodeficiency after prescription of prolonged continuous gamma-interferon therapy in a dose of 500.000-1 million IU[24]. Based on these results, a specially planned controlled trial to assess the efficacy and safety of prophylactic with gamma-interferon immunotherapy in humans with myeloperoxidase deficiency can be initiated. Thus, the purpose

of the study was to evaluate the efficacy and safety of long-term continuous immunotherapy with gamma-recombinant interferon in clinically manifested neutrophil myeloperoxidase deficiency in humans.

Materials and Methods

To achieve the goal of the study, the results of applying long-term continuous immunotherapy with gamma-recombinant interferon in patients of the Institute of Immunology and Allergology with clinically manifested deficiency of phagocytic myeloperoxidase were analyzed for the period from 2009 to 2015. For this time, the diagnosis of myeloperoxidase deficiency in phagocytes was verified in a total of 79 people (31 males and 48 females). The diagnosis was confirmed based on the measurement of myeloperoxidase content in blood neutrophils by laser flow cytometry utilizing the Epics XL device (USA) (Figure 1). The method of indirect immunofluorescence using monoclonal antibodies to myeloperoxidase produced by Beckman Coulter (USA) was applied. These studies were performed in the laboratory of immunodiagnostics of the Institute of Immunology and Allergology of Bogomolets National Medical University (N>90%). The activity of myeloperoxidase of neutrophils was evaluated through the cytochemical method at the Department of Neuroimmunology of the Romadanov Institute of Neurosurgery (N=18-23, conditional units). The diagnosis was made only based on the results of serial studies over several months except for known reasons for secondary immunosuppression. A steady decline of at least one third from the lower limit of the normal was considered to be a deficiency, although the overwhelming majority of patients showed much deeper immunodeficiency. Almost half of the respondents (37 people) had available results of immunological studies of one or more close relatives with a similar phenotype, which allowed stating a family origin of immunodeficiency at least in these cases.

Figure 1. Protocols of laser flow cytofluorimetry in patients with different content of neutrophils myeloperoxidase



Among clinical manifestations, recurring bacterial infections caused by opportunistic microflora prevailed in 64 people or 81% of cases. The most typical causative agents were *S. aureus*, *Str. pneumoniae*, *Str. pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*. Rarely, the failure was caused by *Neisseria spp.*, *E. coli*, and *S. epidermidis*. Mainly, a cocci catalase-negative microflora was observed, especially often – a streptococcal one. It does not correspond to the clinical picture of chronic granulomatous disease with dominating catalase-positive bacteria[1]. Bacterial infections have caused mainly superficial lesions such as rhinitis, sinusitis, tonsillopharyngitis, gingivitis, otitis medial, urethritis, vulvovaginitis, and pyoderma. Deep bacterial infections such as bronchitis, pneumonia, osteomyelitis, arthritis, abscess, meningitis, and sepsis have been reported in only 20% of cases.

Fungal infections were less frequently reported than bacterial ones (48 people, 61% of cases), which does coincide with the report of clinical cases accumulated so far, that candidiasis is the main manifestation of this immunodeficiency[13]. However, mycoses were usually severe, with a tendency for deep tissue lesions and generalization. Candida prevailed among causative agents, mainly C. albicans and less often C. krusei. The lesions of only superficial organs (dermatitis, stomatitis, sinusitis, vulvovaginitis) were recorded in no more than 30% of cases, and in almost 70% of cases, only tissue blocks or combined lesions were involved. Candida enterocolitis, esophagitis, osteomyelitis, arthritis, lung candidiasis, and disseminated candidiasis were observed.

Nearly one of three patients was diagnosed with mold mycoses, which were previously almost unreported concerning myeloperoxidase deficiency. These infections formed mainly superficial lesions like rhinopharyngitis, sinusitis, external otitis, and dermatitis. Only in rare cases, mold mycosis of lungs has been registered, mainly after treatment with glucocorticoids. Penicillium spp., Aspergillus flavus, Aspergillus niger, and Alteraria alternata were mainly detected. Mold mycoses usually occurred in combination with candidiasis. In general, fungal flora prevailed in patients with anamnesis of frequent antibiotics application, and in the clinical picture of individuals, who rarely took antibacterial drugs, bacterial lesions dominated.

The tendency of viral infections development is due to the virucidal properties of myeloperoxidase, which were first recorded for human immunodeficiency virus[25] and cytomegalovirus[26]. Viral infections diagnosed with serum polymerase chain reaction (PCR) were found in 37 people (47% of cases). Rarely, lymphotrophic gamma- and beta-herpesviruses were detected: most often human herpes viruses of types 6 and 7, and less often - Epstein-Barr virus. The most typical manifestations were chronic fatigue syndrome, chronic mononucleosis, and mononucleosis-like syndrome. In 23 out of 37 patients (62% of cases) with chronic virus lifetime organ lesions, mainly neuroinfections were recorded. The predominant clinical form of neuroviral lesions was mono- or multifocal leukoencephalitis, reminiscent of autoimmune demyelinating brain diseases and limbic encephalitis.

Almost 40% of patients performed surgeries for infectious lesions, and in 25% of cases, several surgeries took place during their lifetime. Surgical interventions did not affect the course and consequences of the disease.

In 60% of cases, allergic manifestations of immunodeficiency took place, mainly drug allergy. Atopic dermatitis, rhinitis, conjunctivitis, and food allergies were quite common. Serious allergic lesions like bronchial asthma or anaphylactic shock (25% of cases) were less frequent. In many cases, the episodes were interpreted as allergic, most likely having a pseudoallergic character. Allergic manifestations usually occurred after a period of the infectious syndrome of various duration, but some patients had their first or the only sign of immunodeficiency.

Autoimmune lesions were revealed in almost 45% of patients. In particular, autoimmune thyroiditis caused by the production of autoantibodies to thyroglobulin and thyroperoxidase in more than half of all registered cases prevailed among such lesions. Such an association was reported earlier in the description of the clinical case[12]. Quite often, chronic rheumatic fever was noticed, which is consistent with the frequent release of Str. pyogenes from the oropharynx (one-third of all reported cases). Inflammatory bowel disease, rheumatoid arthritis, and disseminated sclerosis occurred less commonly. These data are consistent with previous reports on how myeloperoxidase deficiency is associated with rheumatism[19], rheumatoid arthritis[11], and multiple sclerosis[18]. However, the relation to nonspecific ulcerative colitis is described for the first time.

Oncological complications such as chronic myelogenous leukemia, promyelocytic leukemia, or breast, lung, and ovarian cancer were registered in no more than 10% of patients. In all individuals, neoplastic manifestations developed in childhood or young age. Patients have had these episodes in the past, received the necessary treatment in specialized centers, and were monitored by the Institute in the phase of long-term sustainable remission of the cancer process. The development of acute myelocytic[27] and promyelocytic leukemia[28], myeloproliferative neoplasia[29], and solid tumors[30] have been previously reported in patients with myeloperoxidase deficiency.

Almost all patients required hospitalization at least twice a year for infectious or non-infectious (allergic, autoimmune) lesions, and at least half of the patients were committed to the hospital more than three times a year. One-third of patients had 2nd or 3rd disability groups at the time of applying to the Institute, for which they were receiving pension benefits.

The immunological study included the evaluation of general blood analysis indicators, subpopulation composition of lymphocytes using laser flow cytometry (Epics XI), and method of indirect immunofluorescence with monoclonal antibodies to CD markers with two or three marks (CD3+, CD3+CD4+, CD3+CD8+, CD3-CD19+, CD3-CD16+CD56+, and CD3+CD16+CD56+). The reagents for the testing were supplied by Beckman Coulter, USA. Phagocytosis was assessed based on data of latex test with the determination of phagocytosis indicator, phagocytic index, number of active phagocytes, and phagocytic blood capacity. Serum concentrations of immunoglobulins of the main classes (M, G, A) were determined from the results of Mancini simple radial immunodiffusion. Concentrations of IgE, IgD, and subclasses of IgG (IgG1, IgG2, IgG3, IgG4) in serum were measured using a solid-phase immunoassay (VectorBEST, the Russian Federation). The Nitro Blue Tetrazolium Reduction Test was repeatedly performed both at the Zabolotny Institute of Microbiology and Virology and the Department of Neuroimmunology of the Institute of Neurosurgery of the Academy of Medical Sciences of Ukraine. Normal values were reported according to obtained results, which is important in differential diagnostics of chronic granulomatous disease.

Patients were prescribed long-term continuous immunotherapy with gamma-recombinant human interferon in a dose of 500 thousand- 1 million IU intramuscular every other day or once per 3 days overnight for consecutive 5-7 months. Of 79 patients, 32 refused the proposed immunotherapy due to financial obstacles or lack of support from other doctors. They formed a control non-treated group. The remaining 47 people received a course of gamma-interferon (the study treated group). Thus, the effect of immunotherapy with the natural course of immunodeficiency was compared. The study and control groups were consisted of each other by age and gender composition, depth of immunodeficiency, and clinical manifestations. The endpoints were the quantitative and qualitative indicators of myeloperoxidase, the number of infectious and noninfectious episodes associated with immunodeficiency, the need for antimicrobial drugs, the number of committals to hospital, and surgical interventions.

The statistical analysis of the obtained information was carried out through structural and comparative analysis using Microsoft Excel software. The obtained data were processed through the method of variation statistics according to the Student's t-criterion with the calculation of significant difference coefficient p (parametric criterion) and the number of Z-signs according to Urbach (non-parametric criterion). The difference was considered significant at $p<0.05$ or $Z<0.05$.

Results and discussion

The activity of neutrophil myeloperoxidase increased significantly in 45 out of 47 patients of the investigated group and only in 12 out of 32 control patients ($p<0.05$ and $Z<0.05$). Complete temporary normalization of myeloperoxidase activity was achieved in 24 patients, and a steady restoration of the normal activity of the enzyme understood as maintenance of the compensated state at least for 3 months after cessation of immunotherapy, took place in 21 people. In the control group, such phenomena occurred in 5 and 3 persons, respectively ($p<0.05$ and $Z<0.05$). The lower was the decrease in myeloperoxidase activity at the moment of the patient's inclusion in the study the faster and more complete was its recovery under the influence of immunotherapy. Myeloperoxidase activity reached its maximum within 2-4 months of immunotherapy.

The content of myeloperoxidase in neutrophils increased significantly in 41 out of 47 patients in the study group, and only in 11 out of 32 control individuals ($p<0.05$ and $Z<0.05$).

Complete temporary recovery of this indicator was achieved in 25 patients, and permanent compensation took place in 16 cases. In the control group, such results were observed in 3 and 4 cases, respectively ($p<0.05$ and $Z<0.05$). The quantitative index reached its maximum only at the end of the immunotherapy course, i.e., after 5-7 months of gamma-interferon intake. At least one-third of patients in the study group had no quantitative changes at all during the first 2-3 months of treatment. No similar correlation between the depth and rate of immunodeficiency recovery and the impact of immunotherapy was registered. The data obtained indicate an expressive positive effect of gamma-recombinant interferon on both qualitative and quantitative indicators of neutrophil myeloperoxidase. Similar reports were not found in the available medical scientific literature: the researches made by Cobourne-Duval et al.[31], Ferreira-Duarte et al.[32], and Harada et al.[33].

The improvement of immune status was associated with a dramatic decrease in the number of infectious and allergic manifestations of immunodeficiency. In particular, a decrease in the number of infectious episodes by 75% and allergic complications by 51% was recorded in the study group, while in the control non-treated group, the number of infectious episodes increased by 4% and allergic complications decreased by 7% ($p<0.05$ and $Z<0.05$). If the positive effect on the infection syndrome was manifested already from 2 months of treatment, the improvement of allergic symptoms became evident only at the end of immunotherapy.

In terms of differences in sensitivity to immunotherapy from different forms of infection syndrome, the following gradation can be pointed out: mold mycosis>bacterial infections>candidiasis>reactivated infections caused by lymphatic herpesviruses.

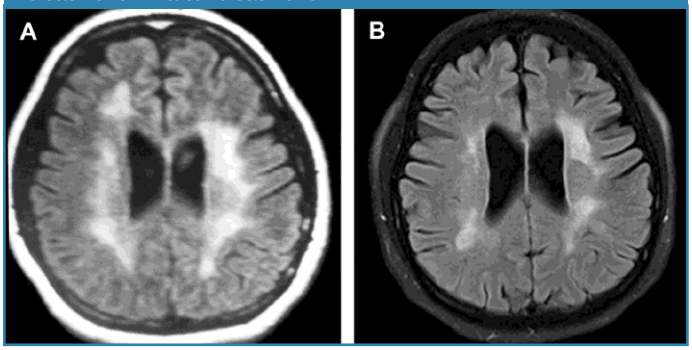
Mold mycoses proved to be sensitive to the immunotherapy, as control of the infection process has been achieved in all registered cases within the first 3-4 months of treatment. Patients who received gamma-interferon no longer needed to take antimicrobials for mold. Only 3 patients with severe immunodeficiency were reported to have relapsed into the mold during the first 6 months after completing immunotherapy. In the control group, the above mentioned positive dynamics was not observed.

Control of candidiasis was also achieved in most cases. In the study group, to eliminate episodes of severe, invasive, or generalized candidiasis was possible within the first 2-3 months of treatment. Clinical manifestations of surface forms of candidiasis were no longer recorded in at least 70% of cases. Microbiological studies revealed candidiasis in no more than 40% of cases in patients with full compensation of candidiasis clinical symptoms. However, in nearly one in three patients with candidiasis, symptoms of stomatitis or vulvovaginitis were persistent, but their severity was mostly reduced by immunotherapy. Most patients did not require fluconazole or other antimycotics for candidiasis. No spontaneous positive dynamics of candidiasis have been observed in the control group, but at least 11 new cases of antimicrobial resistance induction have been reported. Minimum 26 antifungal side effects among control group patients are known, at least nine of which were severe, and three were considered life-threatening.

The number of episodes of bacterial infections decreased by almost 80%, but control microbiological studies indicated that opportunistic flora in the oropharynx and urinary tract remained in at least 50% of cases, despite the elimination or mitigation of clinical symptoms of the disease. In one case, episodes of severe, invasive, or generalized bacterial infections in the background of immunotherapy were recorded. The need for antibiotics was reduced by three times compared to the same period before treatment, and by four times compared to the the control non-treated group. In the control non-treated group, only a slight decrease in the number of bacterial infections episodes against the background of growth of mycosis episodes was noted. It can be attributed to the frequent use of antibacterial drugs.

Episodes of the viremia proved to be the least sensitive to immunotherapy among infection syndromes. The *Epstein-Barr* virus *(*EBV*)* was eliminated from serum in 90% of cases, but mainly after 4-5 months of continuous immunotherapy. The efficiency of blood sanitation with reactivated human herpesvirus 6 (HHV-6) infection reached 60% of cases, while HHV-7 was resistant to immunotherapy in at least half of patients, and such patients needed repeated courses of antiviral treatment. The number of viremia episodes in the control non-treated group remained unchanged ($p<0.05$ and $Z<0.05$). Not a single episode of organ affection of herpesviral etiology was registered during the immunotherapy course. However, at least 7 such cases were recorded in the control non-treated group, 5 of them - nervous system affections (4 - leukoencephalitis, 1 - myelitis), 1 - liver (hepatitis), and 1 - lung (interstitial pneumonitis). Besides, in some patients of the treated group positive dynamics from the side of the neurological lesion were observed (Figure 2).

Figure 2. Positive MR-dynamics of multifocal leukoencephalitis in a patient with myeloperoxidase deficiency while taking gamma-recombinant interferon (own observation). A - before treatment B - after treatment



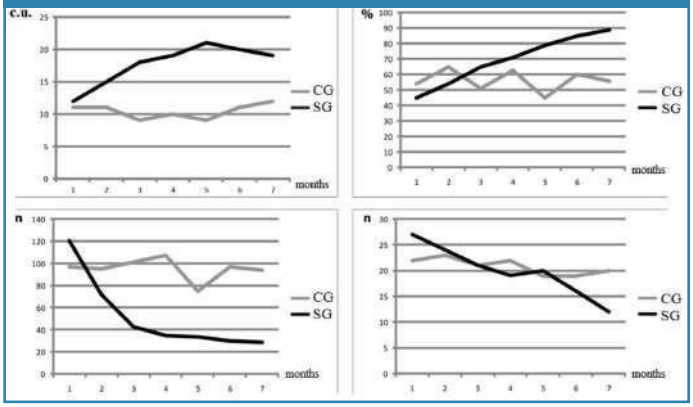
The results obtained by us correspond to the data of clinical studies on the efficacy of recombinant gamma-interferon in another phagocytic primary immunodeficiency - chronic granulomatous disease, in which not myeloperoxidase is affected, but an alternative microbicidal enzyme of neutrophils - NADF-oxidase. Thus, Rex et al.[34] have reported a significant increase in the intensity of digestion of aspergillus hyphae by neutrophils in patients with the chronic granulo-

matous disease during immunotherapy with recombinant human gamma-interferon. According to a study by Bemiller et al.[35], recombinant gamma interferon reduces the number of episodes of serious bacterial and fungal infections by at least 67% in the chronic granulomatous disease. The results of a randomized placebo-controlled clinical trial of Gallin et al.[36] indicate a 72% reduction in the number of severe infection episodes in the chronic granulomatous disease when gamma-interferon is used as a basic immunomodulatory therapy.

The results of a double-blind placebo-controlled randomized clinical trial conducted by the International Chronic Granulomatous Disease Cooperative Study Group with 128 patients confirm that long-term immunotherapy with human recombinant gamma-interferon at a dose of 50 µg/m2 of skin surface three times a week subcutaneously throughout one year dramatically reduces the frequency of severe infections in chronic granulomatous disease in people with good tolerance[37].

Considering the impact of immunotherapy on the allergic syndrome, most expressive it manifested at bronchial asthma being compensated in all respondents, which was a striking contrast to the control group ($p<0.05$ and $Z<0.05$). After cessation of immunotherapy, asthma attacks were no longer observed. Improvement in food allergy was achieved in two-thirds of the allergy cases, and drug allergy, in at least half of the participants (Figure 3). Nevertheless, a significant positive dynamics of atopic dermatitis was registered only in one-third of cases. However, the dermatitis part was not severe and, in most cases, did not critically affect the quality of life of patients. At that, a tendency a better clinical dynamics was observed at more severe allergic syndrome. The reduced serum concentration of IgE was not observed in the vast majority of cases, despite the elimination or mitigation of allergy clinical symptoms, which indicates the implementation of additional compensatory mechanisms. Previously, gamma-interferon demonstrated a positive effect on the allergy course in humans, although the IgE content did not change significantly with immunotherapy[38].

Figure 3. Dynamics of endpoints study during immunotherapy with gamma-recombinant interferon



The upper left corner shows the dynamics of neutrophil myeloperoxidase activity, the upper right corner shows the dynamics of neutrophil myeloperoxidase content, the lower-left corner is the frequency of infectious episodes, and the lower right corner is the frequency of allergic episodes; SG stays for the study treated group and CG stay for the control non-treated group.

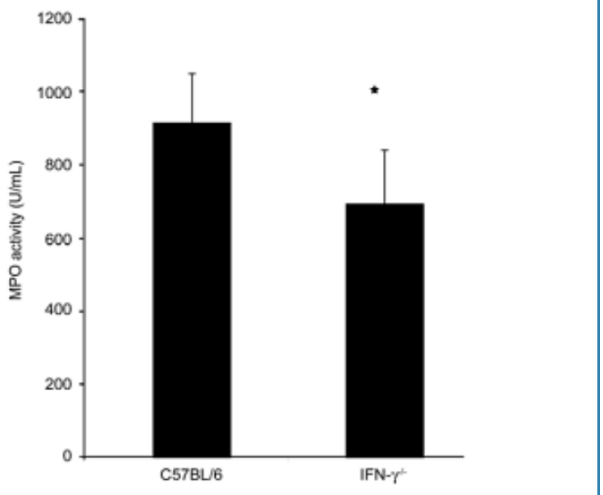
Neither positive nor negative effects of gamma-interferon on the course of autoimmune thyroiditis have been noted, but this complication was mainly mild, and the vast majority of patients did not need L-thyroxin. These data are consistent with the results of previous studies on the absence of negative effects of gamma-interferon drugs on the metabolism of thyroid hormones[39] and the production of antibodies to thyroid antigens[40]. On the other hand, a significant improvement in rheumatoid arthritis and non-specific ulcerative colitis was observed, but conclusions were impossible to make due to quite a small number of observations. At the same time, the benefits of gamma-interferon in rheumatoid arthritis have been reported ($Z<0.05$)[41], which was not stated in the periodical scientific literature.

No patient in the study group had any surgery performed on immunodeficiency disorders, while at least 9 control non-treated patients required surgery ($P<0.05$ and $Z<0.05$). Also, patients who took gamma-interferon did not receive inpatient treatment either during immunotherapy for the first six months after the end of treatment, while less than 17 patients of the study treated group ($P<0.05$ and $Z<0.05$) applied to hospitalization during the same period. One patient from the control non-treated group died from an intestinal abscess and associated bacterial peritonitis after unreasonably prescribing steroids in a residential hospital. At least seven patients in the control non-treated group were found to be treated in the intensive care units, and their lives were saved thanks to the substantial efforts of intensive care specialists. No such cases were observed among the patients of the study treated group.

Positive clinical dynamics were observed in those patients, who had an improvement in myeloperoxidase of phagocytes. All six patients who were not able to influence the microbicide enzyme in immunotherapy demonstrated positive dynamics in terms of the infection syndrome. Sometimes, clinical effects were ahead of laboratory ones, especially in mycoses, which can be attributed to the direct anti-infection effects of gamma-interferon demonstrated in several recent controlled trials[42,43] and the reported series of clinical cases[44], as well as to the known effect of this cytokine on NADPH oxidase of phagocytes[45]. Also, there was a delay at the beginning of clinical manifestations by 3 months on average after the registration of negative laboratory dynamics in the period after completion of immunotherapy. The gamma-interferon is well known to potentiate respiratory explosion associated with the impact on the microbicide system of NADPH oxidase[1], and the production of nitrogen oxide in monocytes/macrophages[46]. The H2O2 required for MPO function comes mainly from the phagocyte NADPH oxidase during the respiratory burst[47]. Ahlin et al.[48] described the ability of gamma-interferon to affect the NADP-dependent microbicide system of neutrophils. However, little information about the influence of this cytokine on the additional system of microbicide of phagocytes mediated by myeloperoxidase exist. In particular, Wolfe[49] showed in an experimental study that myeloperoxidase ac-

tivity was higher in wild mice compared to animals with primary gamma-interferon deficiency caused by knockout of the corresponding gene in lung infection caused by B. pertussis (Figure 4). The obtained clinical data are indirectly consistent with the results of this experimental work.

Figure 4. Decrease in myeloperoxidase in mice with gamma-interferon gene knockout[49]. The C57ID/6 is the line of healthy mice without knockout, IFNγ denote mice knocked out by the gamma-interferon gene



Immunotherapy tolerance was good. The mild transient flu-like syndrome was registered in only 11 out of 47 patients because they used small doses of gamma- interferon. Local allergic symptoms were observed in one per three patients, mainly starting from 2-3 months of immunotherapy. It was recommended to change the places of drug injections. In one case, mild local allergy manifestations did not become an obstacle to continue therapy. No systemic allergic reactions were noted.

Conclusions

The results of the performed controlled trial indicate the obvious benefit of continuous prolonged immunotherapy with human gamma-recombinant interferon in patients with clinically manifested neutrophil myeloperoxidase deficiency. In the overwhelming majority of participants, qualitative and quantitative indicators of neutrophil myeloperoxidase were successfully restored during treatment and were associated with a decrease in the number of episodes of bacterial, fungal, and herpes virus infections. Reduced allergic symptoms, including bronchial asthma, food and drug allergies, and chronic rheumatic fever, reduced need for antibiotics application, commitment to hospitals, and surgical interventions. Comparative analysis of treatment results in both groups showed that gamma-interferon significantly modifies the natural course of the disease, helping to compensate for immunodeficiency.

The proposed immunotherapy can provide not only medical but also social and economic effects, considering the high incidence rate of immunodeficiency in the modern population. The results of this study allow considering the gamma- recombinant human interferon as an effective and safe strategy to prevent phagocytes myeloperoxidase deficiency related to chronic granulomatous disease, congenital osteopetrosis of Type 1, and IL-12/IL-23 pathway defects. Also, additional studies in the outlined direction with a larger number of participants and a more perfect design are required.

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