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Dynamics of Immune Status in Myofibrillar Myopathy with the T341P *DES* Mutation

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ABSTRACT

Introduction. Desminopathy is one of the varieties of myofibrillar myopathy that is caused by a mutation in the *DES* gene. To date, there is a lack of evidence regarding changes in immune status during the natural course of the disease.

The aim of the study was to investigate the dynamics of immune status in myofibrillar myopathy with the T341P *DES* mutation in the heterozygous state.

Materials and methods. The study is based on a long-term observation of a proband with a reliable diagnosis of hereditary desminopathy after the middle of the disease course was reached. An analysis of medical records of the proband's desminopathic father was also carried out. Extended immunological studies were performed by cytometry, enzyme-linked immunosorbent assay, immunoturbidimetry, chemiluminescence immunoassay, and spectrophotometry.

Results. An evident immunosuppression was observed with the progression of the disease. This manifested in the deterioration of the vast majority of immunity parameters, drop of functional (phagocytic, metabolic, oxydative) activity of monocytes and granulocytes, as well as in the increase of the level of IL-6 and IL-8 proinflammatory interleukins. It is found that IgE, IgM, and IgG immunoglobulin levels dropped by factors of 4.4, 3, and 1.6, respectively, but the IgA level increased by a factor of 1.8. A prolonged inflammatory response to skin damage, a reduction in healing rates of affected areas, and incomplete wound healing were detected.

Conclusions. The presented data may potentially be used for assessing the disease pathogenesis and for designing pharmaceutical intervention strategies.

Keywords: Desminopathy, immunity, inflammation, regeneration, monocytes, cytokines.

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INTRODUCTION

Desminopathy is one of the varieties of myofibrillar myopathy that is caused by a mutation in the *DES* gene. The first identified case of familial desmin-related myopathy with c.1021A>C (T341P) *DES* mutation in the heterozygous state is reported in an earlier study [1], which describes clinical signs of the disease with the relevant cardiological and electromyographic parameters, and presents the results of a genealogical analysis. Pathogenesis of desminopathies is a complex multi-level issue [2]. Understanding the role of molecular players involved in muscle homoeostasis and regeneration can help to devise new treatment methods for myodegenerative disorders [3]. Since there are no concrete treatment options yet [4], it is imperative to quickly determine factors that can aggravate or alleviate the progression of the disease. It is known that the immune system is intimately related to muscle regeneration [5], which involves congenital as well as adaptive immune responses [6]. Diverse immune cell and cytokine types play an important role in the regeneration process. However, changes of immune status in patients with myofibrillar myopathy are scarcely reported. Regeneration of skeletal muscles is a complex sequence of events in which monocytes or macrophages coordinate different kinds of cellular interactions and biological processes [7 - 9]. Consideration of behaviour of cells both as individuals and members of integral entities ensures proper understanding of key processes governing the regenerative potential [10-12].

Immunological mechanisms play a broad homoeostatic role in tissue repair and open up new prospects for accelerating muscle growth in chronic diseases [13]. Currently, there is a need to analyze early and intermediate stages of the disease and evaluate new treatment concepts [14]. The aim of the study was to investigate the dynamics of immune status in myofibrillar myopathy with T341P *DES* mutation in the heterozygous state.

MATERIAL AND METHODS

A proband with a reliable diagnosis of hereditary desminopathy (with T341P *DES* mutation in the heterozygous state) was observed on a long-term basis after the middle of the disease course was reached at the age of 41 years. We also analyzed the medical records of the proband's desminopathic father, who died at 49.

All samples of the proband's venous blood for standard follow-up laboratory tests were taken from 9 to 10 am on an empty stomach on the same date of the month for several years. Extended blood immunogram, metabolic activity studies, and phagotest were performed by flow cytometry using the Cytomics FC500 instrument (Beckman Coulter, USA) with Beckman Coulter reagents (USA). The bactericidal activity of blood was determined using the Navios 10/3 flow cytofluorimeter (Beckman Coulter, USA) with BURSTTEST Becton, Dickinson and Company, and BD Biosciences reagents (USA).

C3 and C4 complement components and immunoglobulins (IgA, IgG, IgM) in blood serum were measured by immunoturbidimetry using the AU 5800 instrument (Beckman Coulter, USA) with Beckman Coulter reagents (USA). Serum levels of interleukins (IL) and immunoglobulin E were determined by chemiluminescent immunoassay using the IMMULITE 1000 automatic analyzer (Siemens, Germany). Tumour necrosis factor (TNF-α) and interferon status were determined by enzyme-linked immunosorbent

immunoassay using the Vector-best instrument (Russia) and reagents from the same manufacturer. Level of circulating immune complexes was measured by spectrophotometry using the Thermo MULTISKAN FC instrument (ThermoFisher Scientific Inc., Finland).

RESULTS

The proband and his father with confirmed desminopathy were physically strong for the first three decades of their lives, and rarely had colds. At the same time, the proband had slightly enlarged inguinal, cervical, and submandibular lymph nodes from early childhood until the age of 28. In addition, from the age of 11 to 28, the proband periodically experienced digestive problems, which manifested in liquid stool 1-2 times a day. At 16, he was bitten in the shin by a dog, resulting in a course of rabies vaccine. At the age of 20, the proband got a 2nd degree sunburn with an area of 18%; chest and abdomen were affected. At the same age, the proband was diagnosed with pollinosis, allergic rhinitis, allergy to tree and grass pollen with seasonal exacerbation from June to August. Histamine test was positive for cereal mixture, birch, mugwort, and fathen. The proband's allergy symptoms gradually decreased with age and have almost disappeared by the age of 38. By an odd coincidence, the proband's father has also been vaccinated against rabies by the age of 18 and has also gotlarge sunburn of the skin. The weakness of skeletal muscles in these desminopathic patients appeared at the age of 30. From the age of 38, the proband moved with a cane, and from the age of 40 - with a walking frame. The proband does not take any medications on a regular basis. His father used a wheelchair from the age of 41 and died of lung and heart failure 20 years after the manifestation of the disease.

The comparative dynamics of monocytes and lymphocytes in the blood of the proband and his father with desminopathy is shown in Fig. 1.

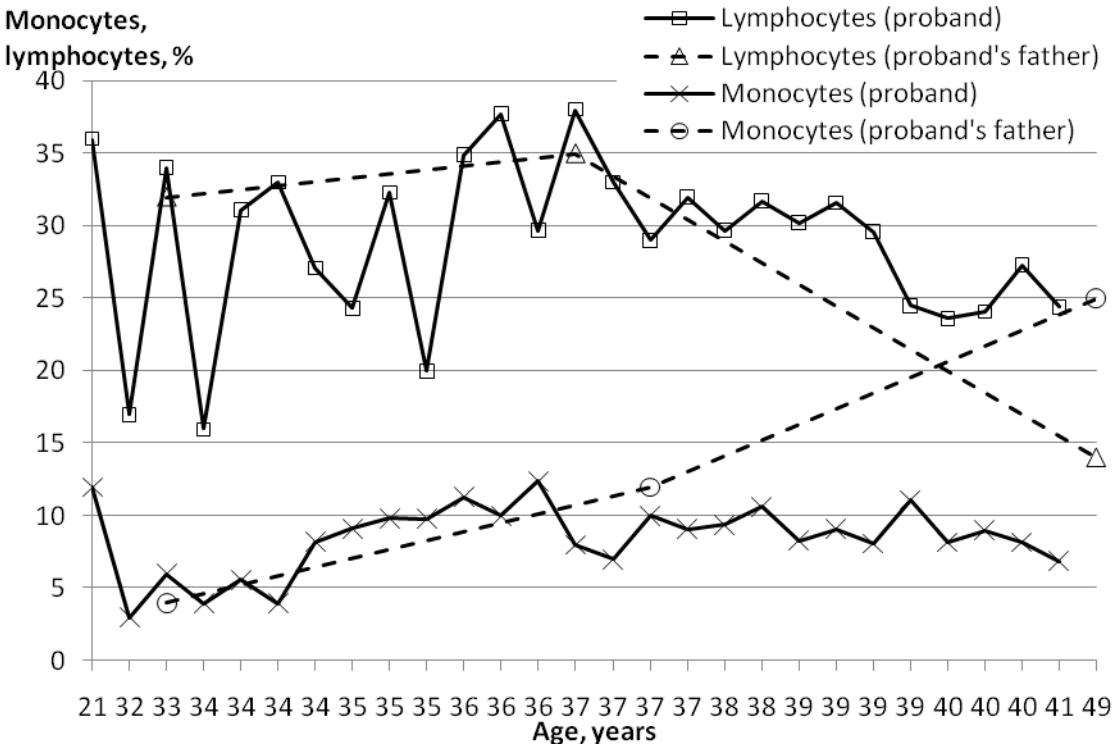

Fig. 1. Dynamics of monocytes and lymphocytes in the blood of the proband and his father with desminopathy *DES* c. 1021A>C (T341P)

The monocyte count in the proband has been within the reference range during the 20 years under study. Fourteen days before the death of the father at the age of 49, his monocyte level was at its maximum of 25%, which is 2 times higher than the norm, and the level of lymphocytes was at its minimum. The lymphocyte pattern shows a gradual decrease starting from the age of 37 in both the proband and his father.

The dynamics of immunoglobulins in the serum of the desminopathic proband is shown in Fig. 2a. Over the past 7 years, IgE, IgM, and IgG have decreased by factors of 4.4, 3, and 1.6, respectively, and IgA has increased 1.8 times. At the same time, at the age of 34, IgE was twice the norm. Over the last 2 years, there has been some stabilization of immunoglobulin levels.

Over the last 3 years, the proinflammatory interleukins IL-6 and IL-8 have increased by factors of 3.4 and 6.9, respectively (Fig. 2b). At the same time, the concentration of IL-6 at the 41st year of the proband's life reached the maximum value of 18.96 pg/ml, which exceeds the norm 2.7 times. The concentration of the anti-inflammatory interleukin IL-4 has simultaneously increased from 0 to 10.7 pg/ml over the last 2 years. Meanwhile, concentrations of interleukins IL-1β and IL-10 are zero. The concentration of TNF-α in the proband during the years under study is normal (<1 pg/ml). The level of interferon IFN-γ is also within the reference range.

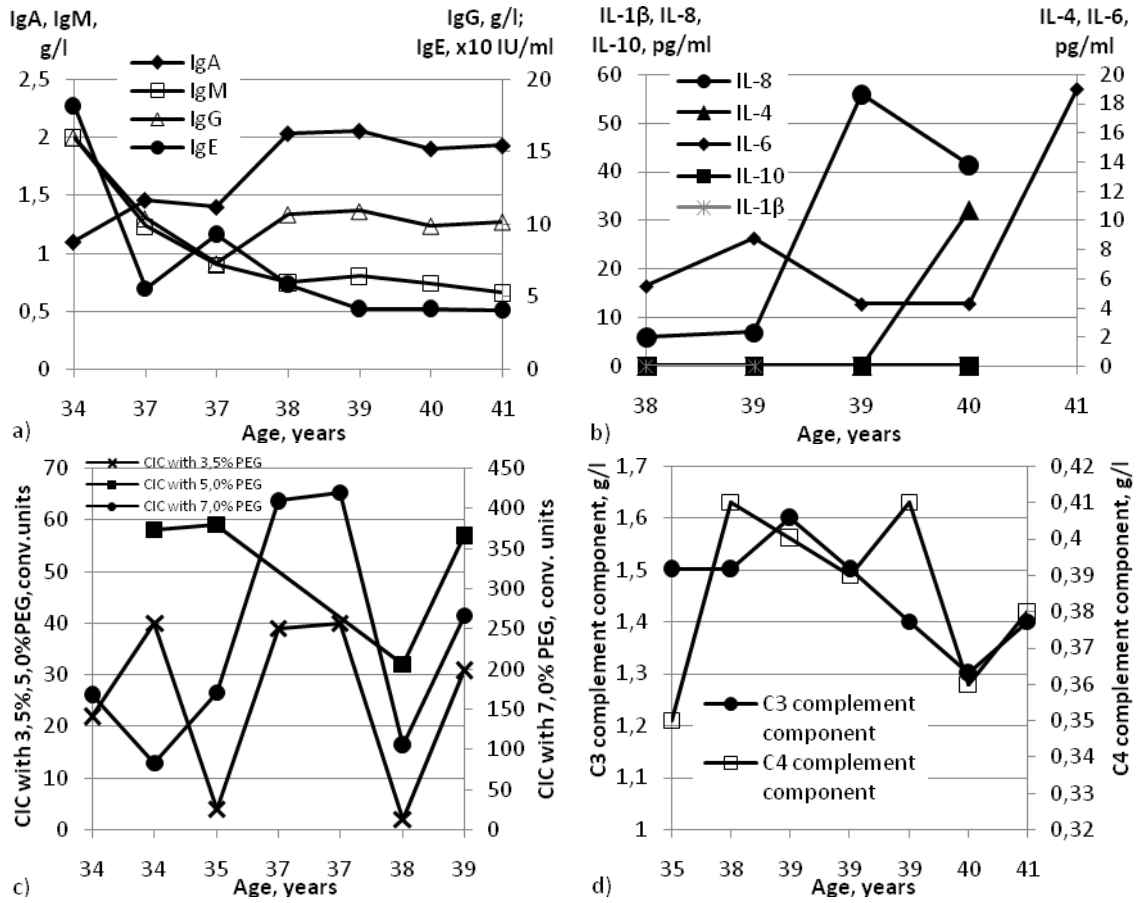

Fig. 2. Dynamics of immunoglobulines (a), interleukins (b), circulating immune complexes (c) and complement components (d) in the blood of the proband with desminopathy *DES* c.1021A>C (T341P)

The dynamics of circulating immune complexes (CIC) with different concentrations of polyethylene glycol (PEG) is shown in Fig. 2c. The greatest changes in levels are observed in CIC with 3.5 % PEG for the proband age ranges of 34-35 and 37-38 years with a decrease10-20-fold, and a subsequent increase by the same number of times. The level of CIC with 5.0 % PEG at 38 years decreased 1.8 times to the lower limit of normal, and then returned to the original value. Between 34 and 37 years of age, there is a 5-fold jump in the CIC with 7.0 % PEG, exceeding the norm by 109-119 conv. units, followed by a 4-fold drop.

When the proband was 35-38, his level of C4 complement component increased by a factor of 1.17 and was at the upper limit of normal for the next 2 years, and even slightly exceeded it (Fig. 2d). The C3 complement component was stable from 35 to 39 years of age, but later its level decreased by a factor of 1.14-1.23. The dynamics of functional activity of monocytes and granulocytes of the proband with desminopathy *DES* c.1021A>C (T 341 P) is shown in Fig. 3.

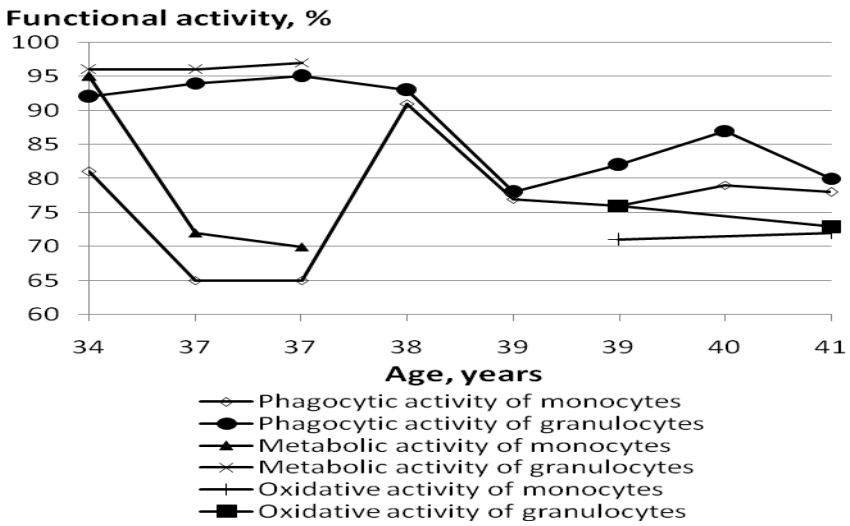

Fig. 3. Dynamics of functional activity of monocytes and granulocytes in the blood of the proband with desminopathy *DES* c. 1021A>C (T341P)

Metabolic activity of monocytes in the age range of 34-37 years decreased by 25 %, shifting to the lower limit of normal, along with the metabolic activity of granulocytes.
Until the proband was 38 years, the phagocytic activity of granulocytes was almost constant and exceeded the norm by 2-5 %, and then there was a sharp decline to values 2-4 % below the norm. Besides, the phagocytic activity of monocytes at 37 was 10% lower than normal values; then, at 38, this parameter grew by a factor of 1.4 and subsequently stabilized at the lower limit of normal.
The oxidative activity of granulocytes decreases and is 73 % when the proband is 41, which is 22% lower than the normal value. The oxidative activity of monocytes is at the lower limit of normal.
At the age of 40, the proband injured his foot because of a short mechanical impact of a treadmill. The dynamics of wound regeneration within one year is shown in Fig. 4a-g.

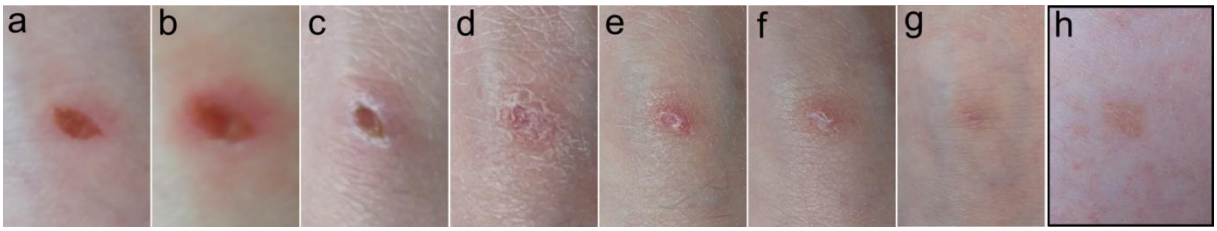

Fig. 4. Dynamics of skin wound regeneration on the foot of the proband with desminopathy *DES* c.1021A>C (T341P) after a - 2 days; b - 6 days; c - 14 days; d - 30 days; e - 2 months.
f - 4 months; g - 1 year; h - another wound 6 years after an injury

Visual assessment of the wound condition showed that even on the 6th day after the injury there is still inflammation (Fig. 4b), and the scab is dissolved only on the 30th day (Fig. 4d). Later on, the wound area gradually decreases. A pale pink spot remains for 1 year after the injury (Fig. 4g).
The proband also has another wound on his foot, received at the age of 35. Its general appearance 6 years after the injury is shown in Fig. 4h. It should be noted that in both cases, a pale pink spot remains after the injury. It is noteworthy that at the age of 41, the proband's father burnt his lower leg; traces of the burn did not disappear even by the age of 49.
Fig. 5 shows the dynamics of parameters of the extended blood immunogram, which was recorded on the same dates of the month in different years of life of the desminopathic proband.

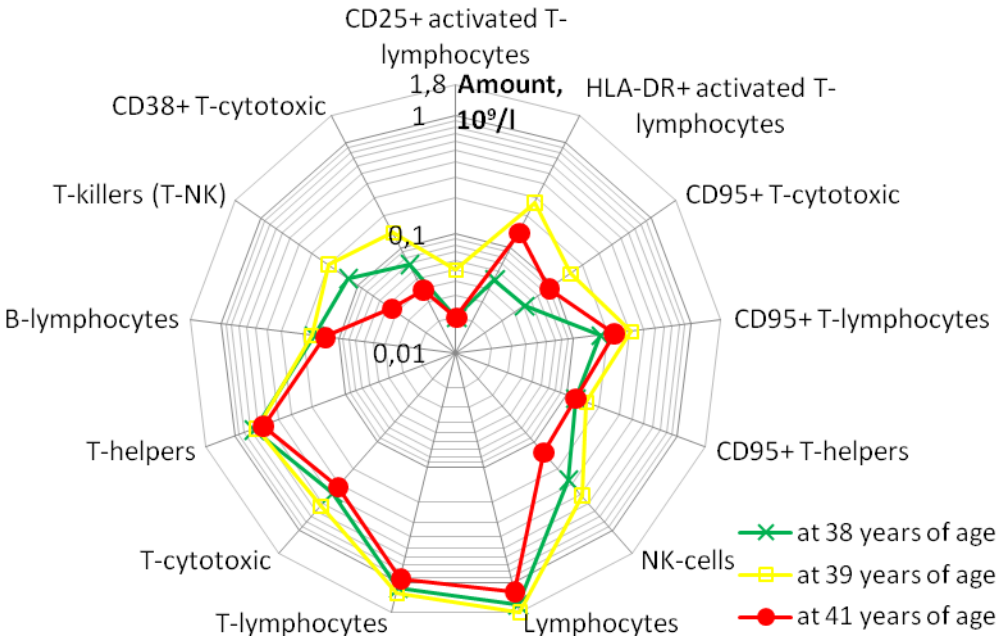

Fig. 5. Dynamics of blood immunogram parameters for the same dates of the month in different years of life of the proband with desminopathy *DES* c.1021A>C (T341P)

The lion part of parameters of blood immunogram of the desminopathic proband have decreased over the last 3 years under study as the middle of the course of the disease approached. The greatest decrease is shown by T-killers (T-NK, 2.8 times), NK cells (2 times), CD38+ T-cytotoxic (1.8 times); the levels of T-lymphocytes, T-cytotoxic, T-helpers, and B-lymphocytes have dropped by a factor of 1.2. Moreover, the level of T-helper cells has

dropped below the lower limit of normal.

This was accompanied by the growth of the following parameters: HLA-DR+ activated T-lymphocytes, T-cytotoxic cells with CD95+ apoptosis marker, and CD95+ T-lymphocytes increased 2.8, 1.8, and 1.3 times, respectively. The values of the proband immunoregulatory index are almost constant for the last 4 years, not exceeding the reference range.

DISCUSSION

An ongoing expression and presence of mutated desmin cannot fail to affect the body's immune system, especially over time. The dynamics of the immune status as observed experimentally, is shown chronologically in Fig. 6 together with notes concerning changes in the body of the proband and his father with desminopathy.

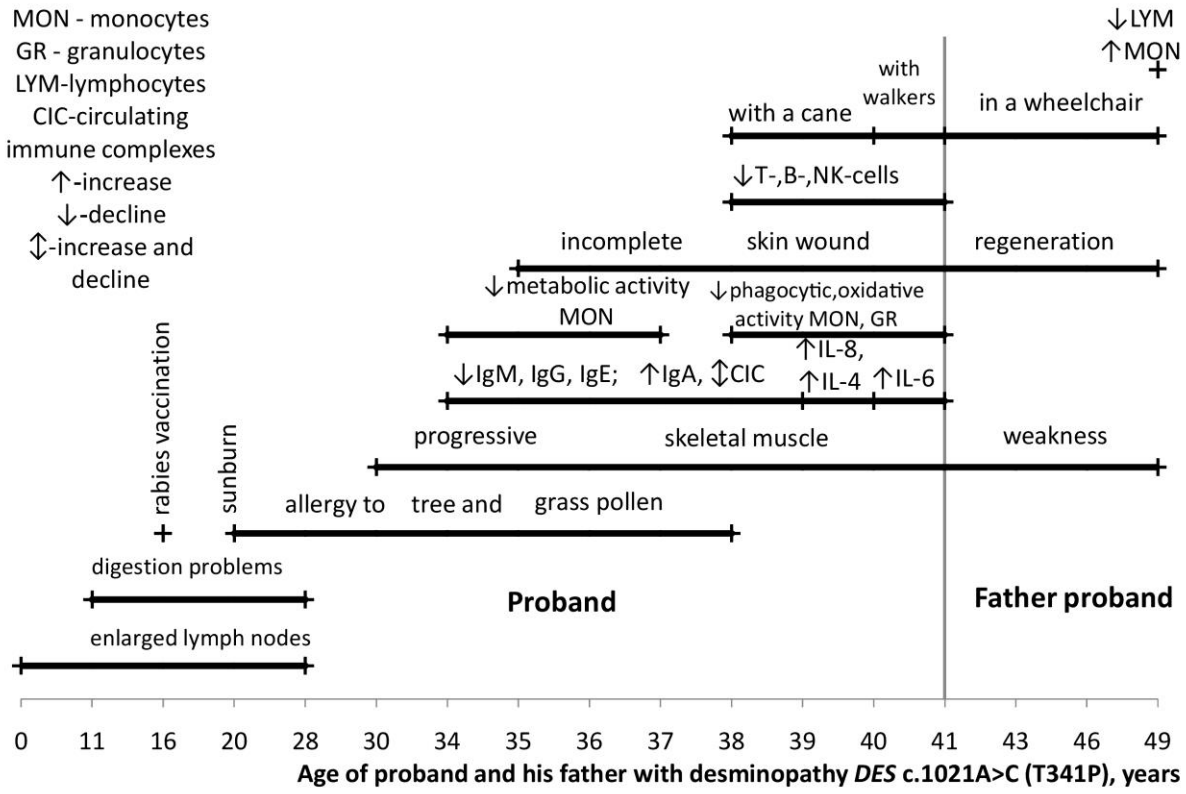

Fig. 6. Dynamics of the immune status shown together with changes in the body of the proband and his father with desminopathy *DES* c.1021A>C (T341P)

Ten years before the manifestation of desminopathy, the proband developed an allergy to tree and grass pollen. It almost ceased to manifest by the age of 38, when the patient began to move with a cane. The decrease in allergic reactions is most likely due to a more than 4-fold decrease in the level of cytokine IgE (Fig. 2a). IgM and IgG concentrations also decreased; conversely, serum IgA, which can cause proinflammatory reactions [15], showed an increase.

It is noteworthy that 2 years before the clinical manifestations of desminopathy in the proband, his lymph nodes, which were enlarged from early childhood, shrunk (Fig. 6). Starting from the age of 34, the metabolic activity of monocytes decreased, and so did, after 4 years, the phagocytic and oxidative activity of monocytes and granulocytes (Fig. 3). It is known that these cells remove debris and apoptotic cells [16]. In addition, monocytes undergo noticeable phenotypic and functional changes after tissue damage that play a critical role in the recovery and regeneration phases [17]. We consider it not accidental that the level of monocytes in the blood of the proband's father was 2 times above the norm 14 days before his death, and that the lymphocyte level dropped 1.7 times below the norm (Fig. 1). In the second quarter of the course of desminopathy, the proband shows multidirectional fluctuations in the levels of circulating immune complexes. The highest and lowest values can differ up to 20 times reaching the upper and lower limits of normal (Fig. 2c).

After the middle of the course of desminopathy is reached, C3 and C4 complement components show a downward trend (Fig. 2d). In fact, a complement is activated when skeletal muscles are damaged and plays a key role during regeneration [18].

The photographs shown in Fig. 4 evidence the duration of inflammatory reaction (Fig. 4b), a decrease in healing rate of affected areas at least 2-fold (Fig. 4d), as well as incomplete wound regeneration 1 year (Fig. 4g) and 6 years after the injury (Fig. 4h). However, at the preclinical stages and in the first quarter of the course of desminopathy, the proband did not experience problems with wound healing. At the same time, his blood sugar and insulin levels are normal. Thus, starting from the second quarter of the course of desminopathy, there is an incomplete regeneration of cutaneous wounds, which occurs both in the proband and his father until the last days of the latter. A similar pathological picture is also likely to occur in the regeneration of muscle tissue of a desminopathic patient, since mechanisms of skin, nerves and muscles regeneration are similar [19].

The growth of the level of proinflammatory interleukins IL-6 and IL-8 in the proband over the last 3 years under

study (Fig. 2b) indicates an increase in inflammation with the progression of desminopathy. Pre-existing inflammatory processes in tissues alter reparative immunity and, eventually, the quality of tissue regeneration [20].

Along with this, the lion part of parameters of the desminopathic proband blood immunogram have decreased by factors of 1.2-2.8 over the last 3 years under study as the middle of the disease course approached (Fig. 5). Additionally, the number of T-cells with the CD95+ apoptosis marker grew 1.3-1.8 times. Even minor imbalances in immune cell populations due to prolonged and successive inflammatory signals in diseased muscles can disrupt cellular dynamics [21].

Summarizing the obtained data on the dynamics of the immune status, we can conclude that there is manifest immunosuppression in the progression of the case of desminopathy under study. The forced permanent regeneration of muscle tissue that occurs in this type of myofibrillar myopathy will change its pattern over time. From a certain point on, the regenerative process will slow down, with a reduction of activation and effectiveness of the immune system.

Previously published data [1] indicate a large number of oncological diseases (n=6) and/or heart diseases (n=5) in this family. Two of these are cases of bladder cancer in men. It is known, meanwhile, that it is overexpression of desmin and vimentin that dysfunction and pathological changes in the smooth muscles of the bladder are associated with [22].

Interestingly, we observed that muscle mass and physical strength of the proband increased for a month after he stopped to brush his teeth with toothpaste (results not published). He continued to brush them twice a day only with water. The proband did not take any medications during this period. After 30 days, the muscles returned to their original condition. Consequently, the cessation of toothpaste led to a change in the oral microbiota, which is known to affect the immune system [23]. Moreover, the correlation between oral microbes and diseases of the nervous system has already been proven, a typical example of which is the Alzheimer's disease [24].

In addition, we observe a noticeable loss of muscle mass in the proband after a night's sleep, which is known to influence muscle recovery [25]. Recent studies also demonstrate the critical role of circadian rhythms and sleep in immune system homoeostasis [26].

To summarize, our opinion is that for patients with rare hereditary diseases, a comprehensive follow-up control should be organized. This will ensure that, by ascertaining changes in the body during the progression of desminopathy, we will get the most complete understanding of the disease pathogenesis, starting from early preclinical stages.

CONCLUSIONS

The reported studies revealed the dynamics of the immune status in myofibrillar myopathy with the T341P *DES* mutation in the heterozygous state. With the progression of desminopathy, there was a evident immunosuppression with a decrease in the vast majority of immune system parameters, functional (phagocytic, metabolic, oxidative) activity of monocytes and granulocytes, as well as an increase in the level of proinflammatory interleukins IL-6 and IL-8. Decreases in IgE, IgM, and IgG (4.4, 3, and 1.6 times, respectively) and an increase in IgA by a factor of 1.8 were found. A long-term inflammatory reaction to skin damage, a reduction of the healing rate of affected areas, and incomplete wound regeneration were observed. The presented data may potentially be used for assessing the disease pathogenesis and for designing pharmaceutical intervention strategies.

ACKNOWLEDGMENTS

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Abbreviations: CIC - Circulating Immune Complexes; Conv. Units - Conventional Units; GR - Granulocytes; IFN - Interferon; Ig - Immunoglobulin; IL - Interleukin; IU - International Unit; LYM - Lymphocytes; MON - Monocytes; NK - Natural Killer; PEG - Polyethylene Glycol; TNF-α - Tumour Necrosis Factor.

REFERENCES

1. Pauls VYu. A Review of Clinical Data of Family Form of Myofibrillar Desmin Myopathy. SRP. 2019; 10(2):130-136.
2. Stöckigt F, Eichhorn L, Beiert T, et al. Heart failure after pressure overload in autosomaldominant desminopathies: Lessons from heterozygous DES-p. R349P knock-in mice. PLoS ONE. 2020; 15(3): e0228913.
3. Cerquone Perpetuini A, Re Cecconi AD, Chiappa M, et al. Group I Paks support muscle regeneration and counteract cancer-associated muscle atrophy. Journal of Cachexia, Sarcopenia and Muscle. 2018; 9:727-746.
4. Ruppert T, Heckmann MB, Rapti K, et al. AAV-mediated cardiac gene transfer of wild-type desmin in mouse models for recessive desminopathies. Gene Ther. 2020; 1-9.
5. Yang W, Hu P. Skeletal muscle regeneration is modulated by inflammation. J.Orthop. Translat. 2018; 13:25-32.
6. Klimczak, A, Kozlowska U, Kurpisz M. Muscle Stem/Progenitor Cells and Mesenchymal Stem Cells of Bone Marrow Origin for Skeletal Muscle Regeneration in Muscular Dystrophies. Arch. Immunol. Ther. Exp. 2018; 66:341-354.
7. Arnold L, Perrin H, De Chanville CB, et al. CX3CR1 deficiency promotes muscle repair and regeneration by enhancing macrophage ApoE production. Nature Communications. 2015; 6:8972:1-12.
8. Lee DE, Bareja A, Bartlett DB, White JP. Autophagy as a Therapeutic Target to Enhance Aged Muscle Regeneration. Cells. 2019; 8:183.
9. Dort J, Fabre P, Molina T, Dumont NA. Macrophages are key regulators of stem cells during skeletal muscle regeneration and diseases. Stem Cells Int. 2019; 2019:1-20.
10. Tierney MT, Stec MJ, Rulands S, Simons B, Sacco A. Muscle Stem Cells Exhibit Distinct Clonal Dynamics in Response to Tissue Repair and Homeostatic Aging. Cell stem cell. 2018; 22 (1):119-127.
11. Sass FA, Fuchs M, Pumberger M, Geissler S, Duda GN, Perka C, Schmidt-Bleek K. Immunology Guides Skeletal Muscle Regeneration. Int. J. Mol. Sci. 2018; 19:835.
12. Duchesne E, Dufresne SS, Dumont NA. Impact of Inflammation and Anti-inflammatory Modalities on Skeletal Muscle Healing: From Fundamental Research to the Clinic. Physical Therapy. 2017; 97(8):807-817.

13. Schiaffino S, Pereira MG, Ciciliot S, Rovere-Querini P. Regulatory T cells and skeletal muscle regeneration. FEBS J. 2017; 284:517-24.
14. Winter L, Staszewska I, Mihailovska E, et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. J. Clin. Invest. 2014; 124:1144-1157.
15. Steffen U, Koeleman CA, Sokolova MV, et al. IgA subclasses have different effector functions associated with distinct glycosylation profiles. Nat Commun. 2020; 11:120.
16. Schreurs M, Suttorp CM, Mutsaers HA, et al. Tissue engineering strategies combining molecular targets against inflammation and fibrosis, and umbilical cord blood stem cells to improve hampered muscle and skin regeneration following cleft repair. Med Res Rev. 2020; 40:9-26.
17. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity. 2016; 44:450-62.
18. Zhang C, Wang C, Li Y, et al. Complement C3a signaling facilitates skeletal muscle regeneration by regulating monocyte function and trafficking. Nat Commun. 2017; 8:2078.
19. Coletti D, Teodori L, Lin Z, Beranudin JF, Adamo S. Restoration versus reconstruction: cellular mechanisms of skin, nerve and muscle regeneration compared. Regen Med Res. 2013; 1:4.
20. Jin RM, Warunek J, Wohlfert EA. Chronic infection stunts macrophage heterogeneity and disrupts immune-mediated myogenesis. JCI Insight. 2018; 3(18): e121549.
21. Bentzinger CF, Wang YX, Dumont NA, Rudnicki MA. Cellular dynamics in the muscle satellite cell niche. EMBO Rep. 2013; 14:1062-1072.
22. Javed E, Thangavel C, Frara N, et al. Increased expression of desmin and vimentin reduces bladder smooth muscle contractility via JNK2. The FASEB Journal. 2020; 34.2:2126-2146.
23. Slocum C, Kramer C, Genco C. Immune dysregulation mediated by the oral microbiome: potential link to chronic inflammation and atherosclerosis. J Intern Med. 2016; 280:114-28.
24. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell. 2018; 9:488-500.
25. Romdhani M, Hammouda O, Chaabouni Y, et al. Sleep deprivation affects post-lunch dip performances, biomarkers of muscle damage and antioxidant status. Biol Sport. 2019; 36(1):55-65.
26. Haspel JA, Anafi R, Brown MK, et al. Perfect timing: circadian rhythms, sleep, and immunity - an NIH workshop summary. JCI Insight. 2020; 5(1): e131487.