Abstract  The expression level of tumor necrosis factor (TNF)-α is elevated in idiopathic inflammatory myopathies and Duchenne muscular dystrophy (DMD), but the precise role of TNF-α is unknown. To elucidate the possible role of TNF-α, we investigated the expression of TNF-α and its receptor in polymyositis (PM), dermatomyositis (DM), and DMD using in situ hybridization (ISH) and immunohistochemistry. We showed that TNF-α mRNA and protein were present in muscle fibers. TNF-α-positive fibers were observed in all cases of PM, DM and DMD, but were rare or absent in neurogenic disorders and normal controls. The proportion of TNF-α-positive fiber showed a significant positive correlation with the proportion of regenerating fibers that were positive for the developmental form of myosin heavy chain (MHC-d). The number of TNF receptor-positive fibers was small. Some muscle fibers expressed both TNF-α and its receptor simultaneously. Our results indicate that TNF-α is produced and expressed by muscle fibers and associated with muscle regeneration.

Keywords  TNF-α · Polymyositis · Dermatomyositis · Duchenne muscular dystrophy · Muscle regeneration

Introduction

Tumor necrosis factor (TNF)-α is a pleiotrophic cytokine and considered to play an important role in the pathogenesis of inflammatory and autoimmune diseases, including rheumatoid arthritis [23], multiple sclerosis [24], and systemic lupus erythematosus [25]. In inflammatory myopathies, TNF-α has been shown to be up-regulated using reverse transcription-PCR [21] and immunohistochemistry [6, 22, 32, 33], but the pathogenic role of this cytokine is poorly understood. TNF-α is considered to activate inflammatory cells and induce major histocompatibility complex class I expression and muscle fiber atrophy in inflammatory myopathies [6]. Thus TNF-α is supposed to play a role in muscle fiber degeneration rather than the regeneration process [15]. In contrast to these observations on inflammatory myopathies, the level of TNF-α has been shown to elevate in the serum and muscle of non-inflammatory myopathies such as Duchenne muscular dystrophies (DMD) [9].

TNF-α is mainly produced by activated macrophages [13, 36]. In addition, non-hematopoietic non-inflammatory cells are also capable of producing TNF-α [13, 36]. For example, myocardium is shown to synthesize and release TNF-α in response to various stresses, such as ischemia and pressure overloading [10]. TNF-α expression on skeletal muscle fiber itself has been proposed by a few authors [20, 33]. However, detailed information is lacking. On the other hand, TNF-α has been regarded as a mediator of protein catabolism in muscle [2, 4]. Administration of TNF-α to rats increases the ubiquitination of skeletal muscle proteins and enhances muscle protein breakdown [7]. Recently, it was suggested that TNF-α is implicated not only in the degeneration phase, but also in the regeneration process in toxin-induced muscle injury in rats, while production of TNF-α in the regenerating phase was considered to derive from activated macrophages [37]. It is thus still unsolved whether TNF-α is produced in the muscle fiber itself, and whether TNF-α has a role in the process of muscle fiber regeneration. This study addresses these unresolved questions utilizing in situ hybridization (ISH) and immunohistochemistry on human muscle biopsy specimens from inflammatory and non-inflammatory myopathies.
ISh for TNF-α mRNA

ISH was performed according to Liang et al. [19]. Four sets of 48-mer sequence of TNF-α were selected from the human TNF-α mRNA sequence. Each antisense and sense oligonucleotides for human TNF-α cDNA corresponded to bases 913–960, 2,233–2,280, 2,383–2,430, and 2,479–2,526. The oligonucleotide probes were labeled with digoxigenin (DIG). The frozen sections were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), washed in PBS, then treated with 0.2 N HCl (20 min), 2,280, 2,383–2,430, and 2,479–2,526. The oligonucleotide probes were labeled with digoxigenin (DIG). The frozen sections were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), washed in PBS, then treated with 0.2 N HCl (20 min), then covered with diaminobenzidine (DAB) for 10 min.

Consecutive sections were also stained with rat monoclonal antibody against the developmental form of myosin heavy chain (NCL-MHC-d, 1:40, IgG1, Novocastra Laboratories Ltd., Newcastle, UK).

Quantification of results

A randomly chosen section containing at least 500 muscle fibers was used to determine the number of fibers positive for TNF-α, TNFR1, TNFR2 and MHC-d. The ratio of TNF-α/MHC-d-double positive fibers to total MHC-d-positive fibers and the ratio of TNF-α-positive/MHC-d-negative fibers to total MHC-d-negative fibers were calculated.

Statistical analysis

The frequencies of TNF-α-positive fibers were expressed as mean ± SD. Statistical analysis was performed by Kruskal-Wallis test. Regression analysis was used to test for a correlation between TNF-α-positive muscle fibers and MHC-d-positive fibers.

Results

Expression of TNF-α mRNA and protein in muscle fiber

TNF-α mRNA was localized within the cytoplasm of muscle fibers as well as infiltrating cells and vessels (Fig. 1). In immunohistochemistry, TNF-α was located both in the cytoplasm and on the surface membrane of muscle fibers and non-muscular cells. A serial section stained with hematoxylin-eosin showed that most of the TNF-α-positive muscle fibers had basophilic cytoplasm and vesicular membrane staining. The frequencies of TNF-α-positive fibers were expressed as mean ± SD. Statistical analysis was performed by Kruskal-Wallis test. Regression analysis was used to test for a correlation between TNF-α-positive muscle fibers and MHC-d-positive fibers.