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# The host defense peptide LL-37 a possible inducer of the type I interferon system in patients with polymyositis and dermatomyositis

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ABSTRACT

The type I interferon (IFN) system has recently been suggested to play important and essential roles in the pathogenesis of myositis. However, a clarification of how type I IFNs could function as triggering factor(s) in the pathogenesis of myositis has yet failed. Through activation of the type I IFN system, the host defense peptide LL-37 carries numerous immunomodulatory properties and is implicated in the pathogenesis of several other autoimmune diseases, including systemic lupus erythematosus (SLE). The expression of LL-37 can be regulated by various endogenous factors including the active form of vitamin D ($25(OH)D_3$). The aim of this study was to explore a potential role of LL-37 in relation to the type I IFN system in patients with polymyositis (PM) and dermatomyositis (DM) and to compare these with SLE patients and healthy controls. We investigated muscle (3 PM, 5 DM) and symptomatic (5 DM) and non-symptomatic (3 PM, 3 DM) skin biopsies from patients with short disease duration and muscle biopsies (3 PM, 1 DM) from patients with long disease duration. Six SLE patients with symptomatic and non-symptomatic skin and five muscle and six skin biopsies from healthy individuals served as controls. Tissue specimens were immunohistochemically stained for LL-37, neutrophils (CD66b), plasmacytoid dendritic cells (BDCA-2), myxovirus resistance protein A (MxA), and macrophages (CD68, CD163). In addition, LL-37 and CD66b double staining was also performed. Serum levels of $25(OH)D_3$ were investigated in PM and DM patients with short disease duration (3 PM, 5 DM) and in 40 healthy controls. We found that the expression of LL-37, BDCA-2 (the major producer of type I IFNs), MxA (an interferon-inducible protein), and macrophages were higher in muscle tissue of PM and DM patients compared to healthy controls. The LL-37 expression was mainly derived from neutrophils. Neutrophils were increased in both symptomatic and non-symptomatic skin of myositis and SLE patients and BDCA-2 was increased in symptomatic DM skin when compared to healthy controls. Moreover, the expression of MxA in symptomatic and non-symptomatic skin of SLE patients was higher when compared to both myositis patients and healthy controls. There was no difference in the expression of LL-37 in skin of myositis and SLE patients compared to healthy controls. All PM and DM patients with a short disease duration had low $25(OH)D_3$ levels compared to healthy controls. In conclusion, the present study supports our hypothesis that LL-37 may activate type I IFNs, which could initiate and perpetuate an inflammatory process. The prolonged exposure of the immune system to type I IFNs may eventually break tolerance and lead to autoimmune myositis.

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## 1. Introduction

Polymyositis (PM) and dermatomyositis (DM) are chronic inflammatory diseases characterized by muscle weakness, fatigue,

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and by skin rash in patients with DM [1]. In both PM and DM, inflammatory cell infiltrates in muscle tissue are commonly present, composed of T-cells, macrophages, and dendritic cells (DCs), including plasmacytoid DCs (pDCs) [2,3]. Different patterns of inflammatory cell infiltrates suggest various molecular pathways may predominate in subsets of myositis.

Recent studies have highlighted an activation of the innate immune system, including type I interferons (IFNs) and IFN-regulated proteins in DM [3–5]. Both IFNα and IFNβ gene expression have been described in muscle biopsies from PM and DM and are correlated to disease activity [6]. The major producer of type I IFNs, the pDCs expressing the BDCA-2 marker, have been found in inflammatory muscle infiltrates and within DM skin lesions [3,4,7–11]. Expression of myxovirus resistance protein A (MxA), regulated by type I IFN, has also been detected in muscle tissue of myositis patients and was significantly higher in patients positive for immune complexes containing anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies and RNA, suggesting activation of the IFN system by immune complexes similarly to what has been reported in patients with systemic lupus erythematosus (SLE) [12]. However, also DM patients without these autoantibodies had increased MxA expression [8].

Another possible mechanism of activation of the IFN system is viral infection, however, the search for viral infections in PM and DM has failed. A third possible inducer of the type I IFN system is through anti-microbial peptides [13]. Cathelicidins comprise one group of anti-microbial peptides of which LL-37 is the only known expressed in humans. LL-37 is produced constitutively or in response to presence of bacteria or their products. Besides its anti-microbial functions, LL-37 also exhibits pro- and anti-inflammatory immunomodulatory roles including an activation of the type I IFN system [14]. LL-37 is involved in the pathogenesis in several autoimmune conditions [15]. The expression of LL-37 can be regulated by various endogenous factors including the active form of vitamin D ($25(OH)D_3$) and short-chain fatty acids [16]. The role of LL-37 in PM and DM has not yet been investigated.

Herein, we postulate that LL-37 may be one inducer of type I IFNs in myositis. We therefore investigated whether LL-37 is expressed in muscle and skin tissue of PM and DM patients in early phase of disease and its relation to pDCs and MxA. As controls we have included symptomatic and non-symptomatic skin of SLE patients, as they are known to express LL-37 and type I IFNs, and muscle and skin samples from healthy controls. In addition, we investigated if serum levels of $25(OH)D_3$ as a possible mechanism on LL-37 expression in PM and DM patients with a short disease duration. Finally, we correlated the expression of these markers to clinical manifestations.

## 2. Material and methods

### 2.1. PM and DM patients

Twelve patients [6 PM (4 definite and 2 probable PM) and 6 DM (4 definite and 2 probably DM)] with a median age of 57 years (range 46–77 years) were included in this study [17,18]. Eight consecutive untreated (except one patient treated 4 months prior biopsy sampling) patients with symptoms ≤ 6 months (median = 3 months, range 1–6 months) who gave consent to a muscle and skin biopsy for research were included. Muscle biopsies from four additional treatment resistant patients with long disease duration (median = 120 months, range 60–216 months) were included as comparators. Autoantibodies were analyzed using protein immunoprecipitation, as previously described [19]. Creatine kinase (CK)-levels and muscle function assessed by functional index (FI) are listed together with detailed clinical information in Supplementary Table 1.

### 2.2. SLE patients

Skin biopsies from six patients with SLE (2 SLE, 2 SLE and discoid LE (DLE), 1 SLE and subacute cutaneous LE (SCLE), and 1 SCLE) with a median age of 62 years (range 37–77 years) were included as comparators. Median disease duration was 84 months (range 24–156 months). Anti-nuclear antibodies (ANA) were analyzed on HEP-2 cells. An ANA titer of at least 1/100 was defined as a positive finding for ANA. The presence of anti-Ro and anti-SSA antibodies was evaluated with ELISA and immunodiffusion was performed as a confirmatory test. For more detailed clinical information see Supplementary Table 1.

### 2.3. Healthy controls

Muscle biopsies from five healthy subjects (median age = 58 years, range 36–69 years) and skin biopsies from six other subjects (median age = 43 years, range 32–51 years) served as controls. None of the controls had any clinical or historical evidence of skin disease, muscle disorder, infection, or other systemic illness.

All patients and controls gave their informed consent and the study was approved by the local ethics committee at Karolinska University Hospital Solna, Stockholm, Sweden.

### 2.4. Muscle biopsies

Muscle biopsies were taken from vastus lateralis or tibialis anterior muscle under local anesthesia utilizing a semi-open technique [20]. At least two muscle biopsies were obtained and evaluated using conventional histopathology and immunohistochemistry staining by an experienced neuropathologist. The biopsies were frozen in liquid nitrogen and stored at −80 °C until sectioned. To confirm unchanged histopathology of the biopsies in the consecutive series of sections, the first and last section were evaluated and stained with Mayer's haematoxylin and eosin.

### 2.5. Skin biopsies

Skin samples were obtained as punch biopsies under local anesthesia by an experienced dermatologist from symptomatic (5 DM) and non-symptomatic (3 DM and 3 PM) skin from eight myositis patients with short disease duration, from symptomatic and non-symptomatic skin from all six SLE patients, and from six healthy controls. The biopsies were frozen in liquid nitrogen and stored at −80 °C until sectioned. Non-symptomatic skin biopsies and biopsies from healthy controls were taken from the gluteal region.

### 2.6. Serum samples

Serum samples were collected at time of biopsy from the eight patients with short disease duration and forty gender and age-matched controls from a population-based randomly drawn control group (median = 55 years, range 37–70 years). The controls were also matched for the month of blood sampling. Serum samples were stored frozen at −80 °C until use.

### 2.7. Immunohistochemistry and immunofluorescence stainings

Biopsies were cut into 7 μm thick sections, placed on chrome gelatine coated slides, air dried for 30 min before they were fixed for 20 min in cold 2% formaldehyde and then washed twice for 5 min in cold phosphate buffered saline. Section slides were kept in −80 °C until use.

Immunohistochemistry stainings were conducted on

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