ARTHRITIS & RHEUMATISM
Vol. 64, No. 2, February 2012, pp 533–541
DOI 10.1002/art.33350
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Immature Muscle Precursors Are a Source of Interferon-β in Myositis

Role of Toll-like Receptor 3 Activation and Contribution to HLA Class I Up-Regulation

Anne Tournadre,[1] Vanina Lenief,[2] Assia Eljaafari,[2] and Pierre Miossec[2]

Objective. **To investigate the production of type I interferon (IFN) by myoblasts and to identify its cell source and the link to Toll-like receptor (TLR) and C-type lectin receptor (CLR) expression and function in myositis biopsy sections.**

Methods. **Production of IFNβ was assessed in cultured myoblasts after stimulation with the TLR-3 agonist poly(I-C) or with cytokines involved in Th1 and Th17 differentiation. Expression of HLA class I molecules by myoblasts was analyzed by fluorescence-activated cell sorting after activation of TLR-3 and IFNβ neutralization. In muscle biopsy samples from patients with polymyositis or dermatomyositis, expression of IFNβ, CD56 (a marker of immature muscle precursors), and HLA class I was analyzed using immunohistochemistry. Inflammatory infiltrates were characterized for the expression of myeloid dendritic cells (DCs), their associated CLRs, and the products of activated DCs, interleukin-12 (IL-12), and IL-23.**

Results. **In cultured myoblasts, stimulation of TLR-3 induced the production of IFNβ when combined with IFNγ and up-regulated the expression of HLA class I molecules, which was decreased after IFNβ blockade. In myositis biopsy tissues, immature muscle precursors overexpressing HLA class I were identified as a source of IFNβ. CLRs associated with myeloid DCs were broadly expressed in inflammatory infiltrates, in association with IL-12 and IL-23, and with immature muscle precursors.**

Conclusion. **Immature muscle precursors may represent a local source of IFNβ and the target of an immune response involving activated DCs associated with the expression of CLRs and of IL-12 and IL-23, which are implicated in T cell polarization. In turn, such local production of IFNβ after TLR-3 activation in the presence of the Th1 cytokine IFNγ may explain HLA class I overexpression in myositis.**

Polymyositis (PM) and dermatomyositis (DM) are inflammatory muscle disorders that lead to destruction of the muscles. Their autoimmune origin is supported by a T cell–mediated myocytotoxicity, the overexpression of class I major histocompatibility complex (MHC) molecules in muscle cells, and the association with autoantibodies. The detection of cytokines involved in Th1 and Th17 differentiation in the lymphocytic infiltrates of PM and DM tissue samples and the association with the migration, differentiation, and maturation of dendritic cells (DCs) (1,2) reflect an ongoing immune response involving activated T cells and DCs. Although still a matter for debate, many studies suggest a main Th1 profile in inflammatory myopathies (3–6).

Aside from the role played by adaptive immunity, the detection of plasmacytoid DCs in muscle tissue, the up-regulation of the type I interferon (IFN) system, and the overexpression of Toll-like receptors (TLRs) suggest a role of the innate immune system (7–11). The connec-

Supported by funding from the Programme Hospitalier de Recherche Clinique Interrégionale to the Centre Hospitalier Universitaire Clermont-Ferrand, the Region Rhône-Alpes, and the Hospices Civils de Lyon.

[1]Anne Tournadre, MD, PhD: University of Lyon, Unit EA 4130 and Edouard Herriot Hospital, Lyon, France, and Centre Hospitalier Universitaire Clermont-Ferrand, Clermont-Ferrand, France; [2]Vanina Lenief, Assia Eljaafari, MD, PhD, Pierre Miossec, MD, PhD: University of Lyon, Unit EA 4130 and Edouard Herriot Hospital, Lyon, France.

Address correspondence to Pierre Miossec, MD, PhD, Department of Immunology and Rheumatology, Hospital Edouard Herriot, 69437 Lyon Cedex 03, France. E-mail: pierre.miossec@univ-lyon1.fr.

Submitted for publication February 15, 2011; accepted in revised form September 8, 2011.

tion between the innate immune system and the adaptive immune response is dependent on the activation of pattern-recognition receptors, such as TLRs and C-type lectin receptors (CLRs), which are expressed by a wide variety of cells, including inflammatory cells as well as epithelial and endothelial cells. DCs and macrophages express a large collection of CLRs, such as DC-SIGN, asialoglycoprotein receptor (ASGPR), and macrophage mannose receptor (MMR), which are more broadly expressed (12). Their stimulation by microbial pathogen-associated molecular patterns delivers activation signals to immune cells, especially to myeloid DCs, leading to the production of cytokines that differentiate and polarize T cells (12). In addition to the immune effectors, many studies have identified a possible role of the muscle cell itself in disease pathogenesis, particularly the immature muscle precursors, through the up-regulation of HLA class I antigens (13,14), activation of the TLR pathway (11), and as a source of autoantigens (15).

To extend our previous work on the expression of TLR-3 in myositis and its regulation by cytokines involved in Th1 and Th17 differentiation (11), we investigated the in vitro production and regulation of type I IFN by cultured normal muscle cells as well as in muscle biopsy tissue. Moreover, in the inflamed muscle infiltrate of myositis, we characterized myeloid DCs and their associated CLRs, which play a critical role in initiating the adaptive immune response, as well as the products of activated DCs, the cytokines IL-12 and IL-23, which are implicated in Th1 and Th17 polarization.

Our results indicate that activation of the TLR-3 pathway induces the production of IFNβ by myoblasts in the presence of the Th1 cytokine IFNγ and increases the expression of HLA class I in part through the induction of IFNβ. In myositis muscle tissue, IFNβ expression was associated with CD56+ immature muscle precursors and with HLA class I overexpression. The CLRs expressed by DCs and macrophages were broadly observed in inflammatory infiltrates, particularly around these immature muscle precursors overexpressing HLA class I and in association with IL-12 and IL-23 cytokines.

PATIENTS AND METHODS

Isolation and culture of human myoblasts. Normal muscle samples were obtained from patients with osteoarthritis who were undergoing hip joint replacement. Informed consent was obtained according to the policies of the local ethics committee. After surgery, muscle samples were immediately placed in sterile phosphate buffered saline (PBS) with antibiotics (penicillin, streptomycin) and washed, and the fat and fibrous tissue were removed. Muscle samples were cut into fragments (1–2 mm^3) and incubated at 37°C for 30 minutes with proteolytic enzymes (1 mg/liter of collagenase [Sigma-Aldrich] and 0.05% trypsin [Invitrogen Life Technologies]). After washing and filtration, a first selection to remove fibroblasts was done by incubating the supernatants in petri dishes for 1 hour at 37°C. Unattached myoblasts were then transferred and cultured in F-10 growth medium (Ham's F-10 nutrient mixture [Lonza] supplemented with 20% fetal calf serum [FCS], 2 m*M* L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin) at 37°C in a humidified incubator containing 5% CO_2. Medium was changed every 4 days.

After 10 days, adherent cells were detached with trypsin (Invitrogen Life Technologies), and myoblasts were purified by positive selection as described elsewhere (16) using CD56 microbeads according to the manufacturer's instructions (magnetic-activated cell sorting; Miltenyi Biotec). Cultured myoblasts with a purity of >95%, as assessed by flow cytometry for the presence of CD56, were used between passages 2 and 8.

Stimulation assays. Cultured muscle cells were grown in 6-well plates (3.5×10^5 cells/well) and stimulated for 48 hours with the following agents: 50 ng/ml of IFNγ (R&D Systems), 50 ng/ml of IL-17A (R&D Systems), and 25 μg/ml of poly(I-C) (InvivoGen). Then, supernatants were collected, and IFNβ protein levels were determined by enzyme-linked immunosorbent assay (ELISA) using a Verikine Human IFNβ ELISA kit according to the manufacturer's instructions (PBL InterferonSource). The assay range for the detection of IFNβ was 25–2,000 pg/ml.

Flow cytometry. Cultured muscle cells were grown in 24-well plates (10^5 cells/well) and stimulated for 48 hours with the following agents: 25 μg/ml of poly(I-C) (InvivoGen) and 50 ng/ml of IFNγ (R&D Systems). During stimulation, cultured myoblasts were incubated with increasing concentrations (10–100 μg/ml) of the neutralizing purified mouse anti-human IFNβ antibody (eBioscience) or with 50 μg/ml of a mouse isotype control.

Cells were harvested using 0.05% trypsin (Invitrogen Life Technologies), washed with PBS containing 5% FCS, resuspended in 50 μl of PBS with 5% FCS, and incubated for 20 minutes at 4°C with fluorescein isothiocyanate–conjugated monoclonal antibody against HLA–A/B/C class I (BD Biosciences). Then, the cells were washed 2 times, resuspended in 250 μl of 1% paraformaldehyde, and analyzed using a FACScan instrument. The results were expressed as the average fluorescence intensity of the entire population of cells.

Patients, controls, and muscle biopsies. Muscle biopsy specimens were obtained from 8 patients with myositis (6 women and 2 men; 5 with PM and 3 with DM). Patients with PM and DM had disease that met the Bohan and Peter criteria (17), with characteristic inflammatory lesions observed on muscle biopsy. All patients were untreated prior to muscle biopsy. At the time of muscle sampling, the median age of the patients was 47 years (range 20–67 years). The median time from diagnosis until muscle biopsy was 11 months (range 1–36 months). The presence of anti–PM-Scl, anti–PL-7, and anti-SSA/SSB autoantibodies was recorded in 3 patients.

Muscle tissues from 4 otherwise healthy subjects (2 women and 2 men) who were undergoing joint replacement surgery for osteoarthritis and from 1 patient with a metabolic

myopathy (McArdle's disease or glycogen storage disease type V) were used as controls. For immunohistochemistry studies, tissues were snap-frozen in liquid nitrogen and stored at –80°C until further use.

Immunohistochemistry. Frozen sections of muscle tissues (10 μm) were thawed, fixed in acetone for 10 minutes, air-dried, and then rehydrated in PBS. Immunohistochemical staining was performed for the detection of CLRs (DC-SIGN, ASGPR, and MMR), CD11c+ myeloid DCs, blood dendritic cell antigen 2 (BDCA-2)–positive plasmacytoid DCs, CD68+ macrophages, HLA class I antigens, and cells producing IL-12, IL-23, and IFNβ. Immature muscle precursors were identified using immunostaining for CD56 as previously described (15,18,19). The use of serial sections was required to simultaneously investigate the expression of all of the selected markers.

Serial muscle sections were incubated for 45 minutes with the following primary antibodies: 5 μg/ml of mouse monoclonal anti–DC-SIGN (IgG2b; Dendritics), 5 μg/ml of mouse monoclonal anti-ASGPR (IgG1; Dendritics), a 1:100 dilution of mouse monoclonal anti-MMR (IgG1; Dendritics), 25 μg/ml of mouse monoclonal anti–IL-12 (IgG1; Abcam), 5 μg/ml of rabbit polyclonal anti–IL-23 (IgG; Abcam), a 1:50 dilution of mouse monoclonal anti-CD11c (IgG1; BD Biosciences), a 1:500 dilution of mouse monoclonal anti-CD68 (IgG1; Dako), 5 μg/ml of mouse monoclonal anti-IFNβ (IgG1; R&D Systems), 1 μg/ml of mouse monoclonal anti-CD56 (IgG1; Novocastra), 4 μg/ml of mouse monoclonal anti–HLA class I (IgG2a; Immunotech), and 10 μg/ml of mouse monoclonal anti–BDCA-2 (IgG1; Dendritics). In control experiments, rabbit IgG or matched mouse IgG isotype was applied at the same concentration as the primary antibodies. After washing, the sections were incubated for 15 minutes with biotinylated anti-mouse and anti-rabbit immunoglobulins, followed by a 15-minute incubation with streptavidin–peroxidase complex and 3,3′-diaminobenzidine chromogen solution (Dako). The sections were then counterstained with Mayer's hematoxylin.

Evaluation and semiquantitative scoring of the immunohistochemical staining. Expression of DC-SIGN, ASGPR, MMR, CD11c, CD68, IL-12, and IL-23 was scored as previously described (8). A scale of 0–4 was used, where 0 = no positive cells, 1 = few positive cells, 2 = scattered positive cells, 3 = many scattered positive cells or, alternatively, many positive cells in at least 2 infiltrates, and 4 = some or many scattered positive cells plus many positive cells in at least 2 infiltrates.

The proportion of muscle fibers expressing IFNβ, CD56, and HLA class I were also scored as described previously (8). A scale of 0–5 was used, where 0 = no positive fibers, 1 = 0–20% positive fibers, 2 = 21–40% positive fibers, 3 = 41–60% positive fibers, 4 = 61–80% positive fibers, and 5 = 81–100% positive fibers.

Statistical analysis. Data are presented as the mean ± SEM. The nonparametric Mann-Whitney U test was used to compare groups. P values less than 0.05 were considered significant.

RESULTS

IFNβ-producing capacity of cultured myoblasts. We analyzed in vitro the production of IFNβ by normal cultured myoblasts after 48 hours of stimulation with the TLR-3 ligand poly(I-C), the Th1 cytokine IFNγ, and the Th17 cytokine IL-17A. Human myoblasts did not constitutively produce detectable levels of IFNβ (<25 pg/ml, the detection limit of the ELISA kit). Moreover, stimulation of myoblasts with IL-17A or IFNγ alone had no effect on IFNβ production (Figure 1). Stimulation with poly(I-C) alone (TLR-3 ligand) induced very weak production of IFNβ (mean ± SEM 28.5 ± 10.9 pg/ml) (Figure 1). When poly(I-C) was combined with IFNγ, the production of IFN was significantly increased (mean ± SEM 39.3 ± 4.5 pg/ml; $P < 0.05$ versus unstimulated myoblasts) (Figure 1).

Increased HLA class I expression after stimulation of TLR-3 pathway partly through IFNβ induction. HLA class I overexpression is a classic marker of pathology in myositis and could be the direct effect of type I IFN (20,21). We investigated in cultured myoblasts the effect of TLR-3 activation on HLA class I expression through the induction of IFNβ. HLA class I expression was assessed by flow cytometry in cultured myoblasts after 48 hours of stimulation with the TLR-3

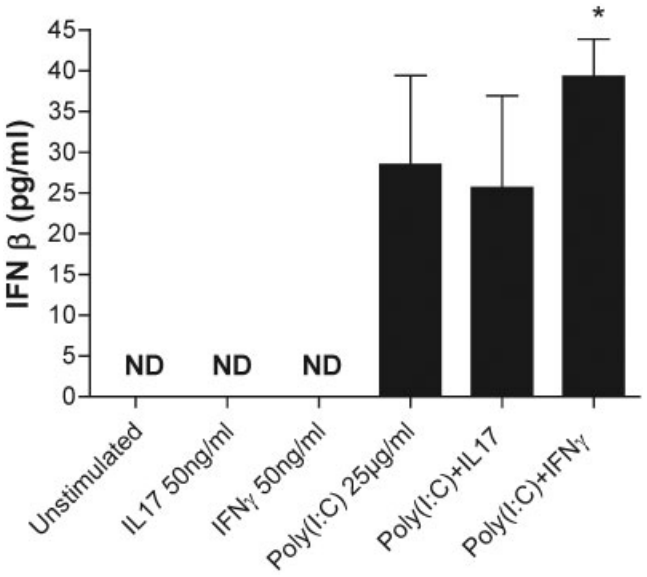

Figure 1. Interferon-β (IFNβ) production in normal cultured myoblasts stimulated with the Toll-like receptor 3 (TLR-3) agonist poly(I-C) and with cytokines involved in Th1 and Th17 differentiation. Cultured myoblasts were stimulated for 48 hours with 25 μg/ml of the TLR-3 agonist poly(I-C), 50 ng/ml of interleukin-17 (IL-17), or 50 ng/ml of IFNγ. IFNβ protein production was determined in supernatants by enzyme-linked immunosorbent assay (ELISA). Values are the mean ± SEM of 5 different myoblast cultures. ELISA results below the level of sensitivity (25 pg/ml) are reported as not detected (ND). * = $P < 0.05$ versus unstimulated myoblasts.

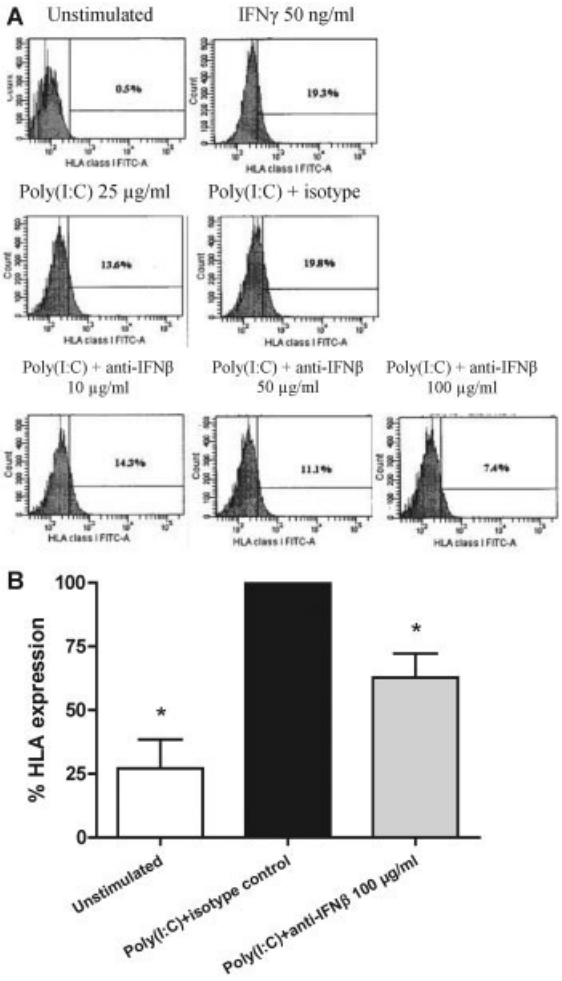

Figure 2. Effect of interferon-β (IFNβ) neutralization on poly(I-C)–induced HLA class I expression by normal skeletal myoblasts. Cultured myoblasts were analyzed by flow cytometry for the expression of HLA class I after stimulation for 48 hours with 25 μg/ml poly(I-C) either alone or in combination with increasing concentrations (from 10 μg/ml to 100 μg/ml) of neutralizing anti-human IFNβ monoclonal antibody or with 50 μg/ml of an isotype control. The expression of HLA class I induced after stimulation with IFNγ (50 ng/ml) is shown as a positive control. Percentages represent the average of cells over the defined fluorescence intensity. **A,** Induction of HLA class I expression after stimulation with poly(I-C) and IFNγ. Increasing concentrations of neutralizing anti-IFNβ monoclonal antibody decreased, in a dose-dependent manner, the expression of HLA class I induced by poly(I-C). **B,** Expression of HLA class I by unstimulated myoblasts or myoblasts stimulated with poly(I-C) in combination with neutralizing anti-IFNβ or isotype control antibodies. HLA class I expression induced by the combination of poly(I-C) and the isotype control was decreased by 38.2% after IFNβ neutralization. Values are the mean ± SEM of 4 different myoblast cultures. $* = P < 0.05$ versus myoblasts stimulated with poly(I-C) and isotype control.

ligand poly(I-C) or with IFNγ, which is known to be a strong inducer of HLA class I (22), either alone or in combination with neutralizing anti-human IFNβ monoclonal antibody. Stimulation with poly(I-C) and IFNγ induced strong expression of HLA class I as compared with unstimulated myoblasts (Figure 2A). IFNβ blockade with increasing concentrations of neutralizing anti-IFNβ monoclonal antibody decreased the TLR-3–induced-HLA class I expression in a dose-dependent manner (Figure 2A). The expression of HLA class I induced by poly(I-C) combined with an isotype control was decreased by 38.2% ($P < 0.05$) after IFNβ neutralization (Figure 2B). These results indicate that HLA class I up-regulation induced by TLR-3 stimulation was dependent in part on IFNβ production.

Identification of immature muscle precursors as a source of IFNβ in myositis muscle tissue. Recent studies have suggested a role for the type I IFN pathway in the pathogenesis of PM and DM (7–10). To define its mechanism of production and its cell source in

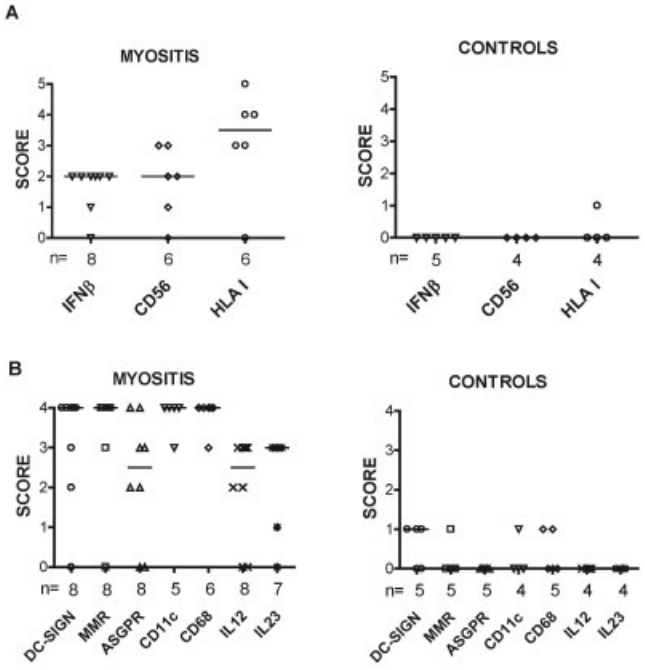

Figure 3. Immunohistochemical staining of muscle biopsy samples from patients with myositis and from control subjects for interferon-β (IFNβ), CD56, and HLA class I, as well as for DC-SIGN, macrophage mannose receptor (MMR), asialoglycoprotein receptor (ASGPR), CD11c, CD68, interleukin-12 (IL-12), and IL-23. **A,** Semiquantitative analysis of muscle fibers expressing IFNβ, CD56, and HLA class I, using a scale of 0–5 (see Patients and Methods for details). **B,** Semiquantitative analysis of cells expressing DC-SIGN, MMR, ASGPR, CD11c, CD68, IL-12, and IL-23, using a scale of 0–4 (see Patients and Methods for details). Numbers on the x-axis are the number of muscle samples analyzed. Each data point represents a single sample; horizontal lines show the median.

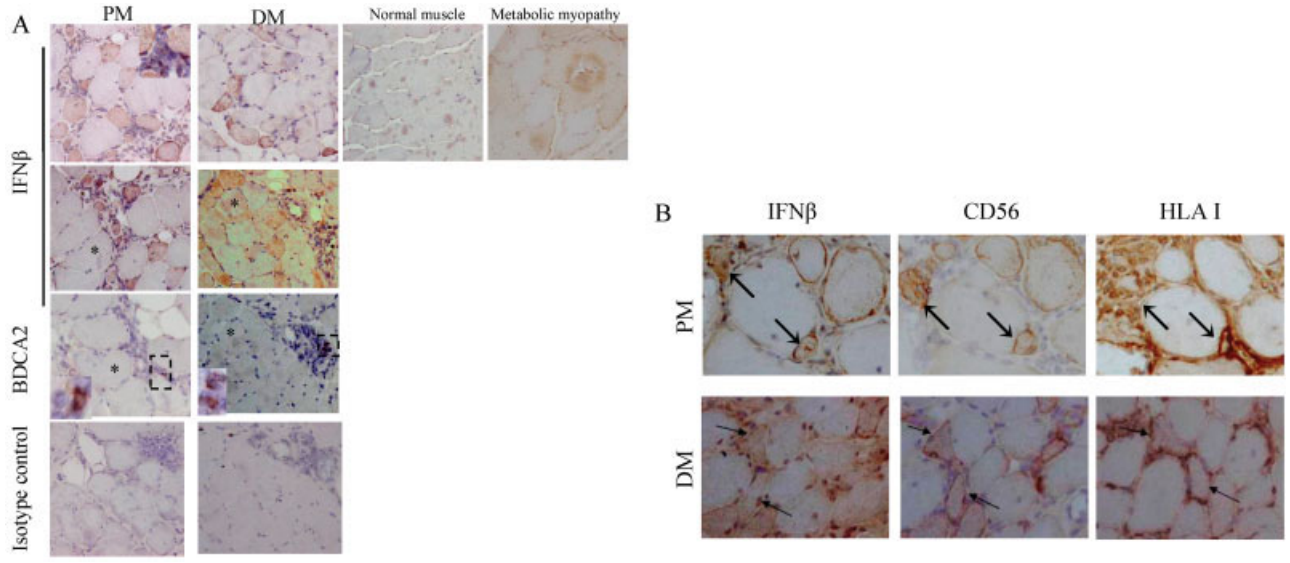

Figure 4. Expression of interferon-β (IFNβ) in muscle biopsy samples from patients with polymyositis (PM) and patients with dermatomyositis (DM). **A,** In DM and PM muscle samples, immunostaining with anti-IFNβ antibody (brown) showed many muscle fibers expressing IFNβ, with endomysial localization in PM samples and perivascular localization in DM samples. Rare IFNβ-producing cells were detected in inflammatory infiltrates (**inset** in the PM sample at top left). Serial PM and DM muscle biopsy sections were stained with antibodies against IFNβ and blood dendritic cell antigen 2 (BDCA-2). Consistent with the rare expression of IFNβ in mononuclear cells, scarce BDCA-2+ plasmacytoid dendritic cells were detected in mononuclear inflammatory cells (**insets**). Boxed areas in BDCA-2–stained sections are shown at higher magnification in the insets. **Asterisks** mark the same muscle fibers in serial sections of muscle stained with IFNβ and with BDCA-2. Control staining was performed using mouse IgG1 isotype. Original magnification × 200. **B,** Serial muscle biopsy sections from a patient with PM and a patient with DM were stained with antibodies against IFNβ, CD56, and HLA class I. Muscle fibers positive for IFNβ also expressed high levels of CD56 and HLA class I antigens (**arrows**). Original magnification × 200. In samples of normal muscle and muscle with metabolic (noninflammatory) myopathy, no muscle fibers were positive for IFNβ (**A**), CD56 (Figure 6C), or HLA class I (Figure 6C).

myositis, we examined IFNβ expression in inflamed and control muscle samples by immunohistochemistry. Positive staining for IFNβ was observed in 7 of 8 myositis patients (5 with PM and 2 with DM) (Figure 3A). IFNβ expression was observed specifically in samples from PM and DM patients, and no positive staining was detected in muscle samples from healthy subjects or from the patient with noninflammatory myopathy (Figure 3A).

In myositis samples, weak expression of IFNβ was detected in inflammatory infiltrates, whereas strong staining was seen in some muscle fibers; this was characterized by a small size, with a perifascicular pattern in DM patients and an intrafascicular pattern in PM patients (Figure 4A). This pattern of rare expression of IFNβ in inflammatory cells was consistent with the rare detection of BDCA-2+ plasmacytoid DCs, the classic major producer of type I IFN (Figure 4A).

Staining of serial myositis muscle sections with antibodies to IFNβ, CD56 (a marker of immature myoblasts [15,18,19]), and HLA class I was performed in 5 patients (4 with PM and 1 with DM). Muscle fibers strongly positive for IFNβ were identified as immature myoblast precursors, expressing CD56 and HLA class I (Figure 4B), in 4 of 5 patients (3 with PM and 1 with DM). These observations strongly implicate immature muscle precursors, rather than plasmacytoid DCs, as the local source of type I IFN production in myositis. We used serial sections because we were not able to perform double staining of muscle fibers with CD56 and IFNβ, since primary antibodies of different species or directly labeled primary antibodies should be used to exclude the possibility that the secondary antibody binds to the same primary antibody. Unfortunately, directly labeled first antibody against IFNβ was not available, and we were unable to obtain positive staining using a goat anti-CD56 antibody as primary antibody and a biotinylated donkey anti-goat antibody as secondary antibody.

Localization of inflammatory infiltrates with CLR-expressing cells around immature muscle fiber precursors. Immunohistochemistry was performed in muscle tissues from patients with PM and DM to investigate the infiltrate around immature muscle fiber precursors, focusing on the expression of DC-SIGN, MMR, and ASGPR, the main CLRs associated with

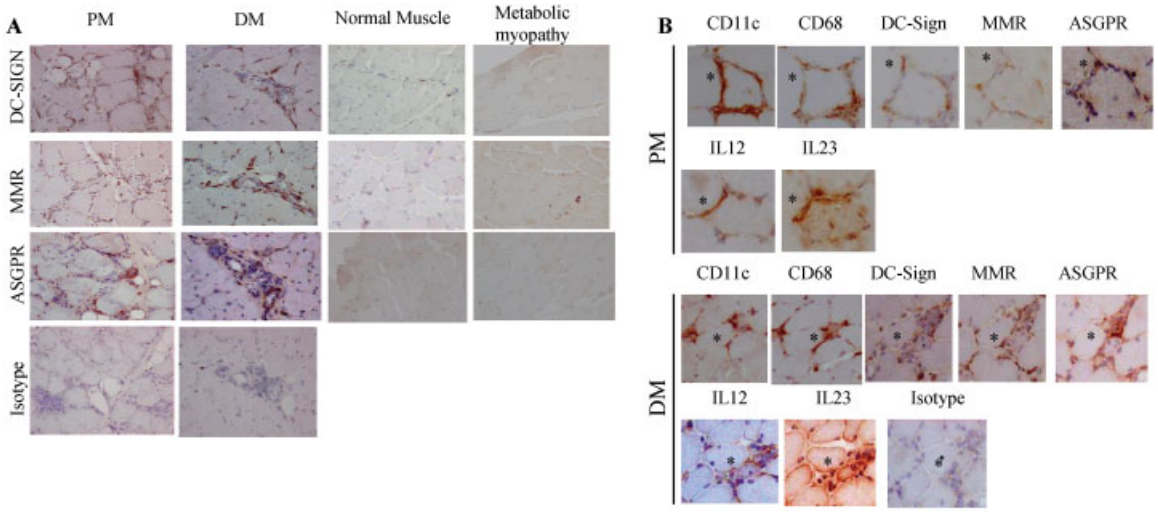

Figure 5. Expression of the C-type lectin receptors (CLRs) DC-SIGN, macrophage mannose receptor (MMR), and asialoglycoprotein receptor (ASGPR) in muscle biopsy samples from patients with polymyositis (PM) and patients with dermatomyositis (DM). **A,** In contrast to the sections of normal muscle and muscle from a patient with metabolic myopathy, the expression of DC-SIGN, MMR, and ASGPR was broadly detected in both PM and DM. CLR expression was observed mainly in cells from inflammatory infiltrates, with a predominantly endomysial localization in PM and perivascular localization in DM. Original magnification × 200. **B,** Serial muscle biopsy sections from patients with PM and DM were stained with antibodies against CD11c, CD68, DC-SIGN, MMR, ASGPR, IL-12, and IL-23. The presence of CLRs was associated with the presence of cells of dendritic cell/macrophage lineage, which were positive for CD11c and CD68, and with the detection of interleukin-12 (IL-12) and IL-23 cytokines. **Asterisks** mark the same muscle fibers in serial sections from the PM patient and the DM patient. Results are representative of serial sections from 5 PM patients and 5 DM patients. Original magnification × 200.

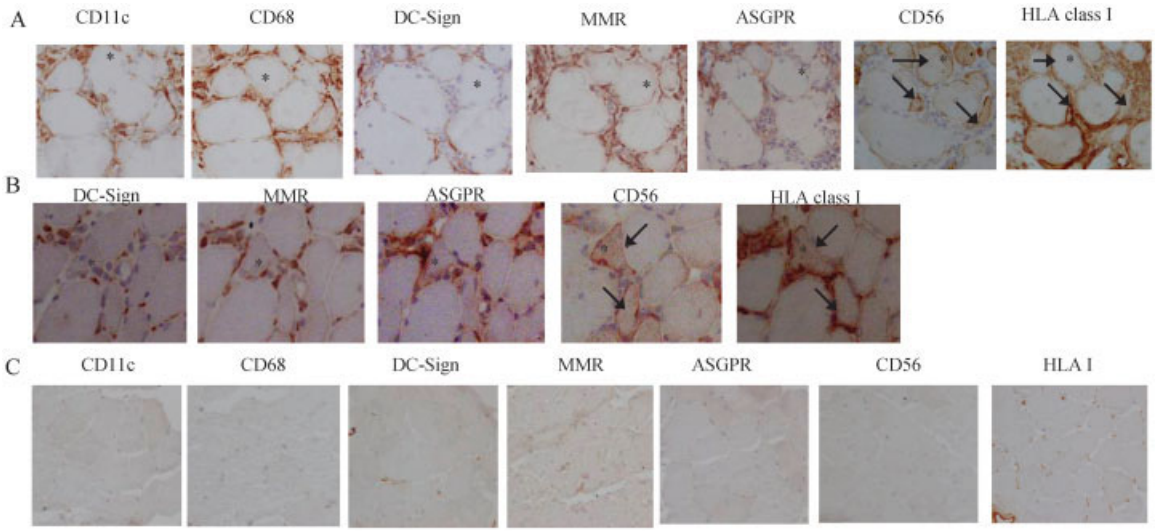

Figure 6. High levels of cells expressing the C-type lectin receptors (CLRs) DC-SIGN, macrophage mannose receptor (MMR), and asialoglycoprotein receptor (ASGPR) around immature myoblast precursors expressing the muscle cell regeneration marker CD56 and HLA class I antigen. Serial muscle biopsy sections from **A,** a patient with polymyositis (PM), **B,** a patient with dermatomyositis (DM), and **C,** a healthy control subject were stained with antibodies against CD11c, CD68, DC-SIGN, MMR, ASGPR, CD56, and HLA class I as indicated. In the tissues from myositis patients, inflammatory infiltrates consisting of dendritic cells and macrophages expressing CLRs were preferentially detected around muscle fibers expressing high levels of CD56, a marker of immature myoblasts and of muscle cell regeneration. Cells expressing CD56 antigens also expressed high levels of HLA class I antigens (**arrows**). No positive staining was observed in normal muscle sections. **Asterisks** mark the same muscle fibers in serial sections from the PM patient and the DM patient. Results are representative of serial sections from 5 PM patients and 5 DM patients. Original magnification × 200.

DCs and macrophages. The expression pattern was compared with that of normal and noninflammatory metabolic myopathic muscle tissues. DC-SIGN and MMR proteins were broadly expressed in all patients with myositis except for 1 patient with DM (Figure 3B). ASGPR expression was detected in 6 of 8 myositis patients (4 with PM and 2 with DM) (Figure 3B). This staining was observed in inflammatory infiltrates with an endomysial pattern in PM samples and a perivascular pattern in DM samples (Figure 5A). In contrast, rare CLR-expressing cells were detected in normal and noninflammatory myopathic muscle tissue, and the grade did not exceed 1 (Figures 3B and 5A).

To better characterize the expression of CLRs in myositis tissue, single-step immunostaining for CLRs, CD11c, CD68, IL-12, and IL-23 was performed in serial sections. Expression of DC-SIGN, MMR, and ASGPR in inflammatory infiltrates was associated with the presence of CD11c+ myeloid DCs and CD68+ macrophages and with the expression of IL-12 and IL-23 cytokines in 5 patients (4 with PM and 1 with DM) (Figure 5B). The presence in inflamed muscle of CLRs expressed by DCs and macrophages, in association with IL-12 and IL-23 cytokines involved in the differentiation of Th1 and Th17 cells, strongly suggests an ongoing immune process involving activated T cells and DCs.

To further investigate whether the muscle cell itself could contribute to the immune response in myositis tissue, immunohistochemistry for CD56 and for HLA class I antigens known to be overexpressed in myositis (14) was performed in serial muscle biopsy sections from 5 patients in addition to CLRs, CD11c, and CD68 immunostaining. In 5 myositis biopsy samples (4 with PM and 1 with DM), the strong positive staining for CD11c, CD68, DC-SIGN, MMR, and ASGPR detected in inflammatory infiltrates was associated with the presence of CD56+ and HLA class I+ muscle fibers (Figures 6A and B). The proportion of CD56+ fibers was scored 1 in 1 patient, 2 in 2 patients, and 3 in 2 patients (Figure 3A). No positive staining was observed in normal muscle tissue (Figure 6C). These results suggest a local immune response focused on immature muscle precursors expressing CD56 and HLA class I antigens in inflamed muscle of PM and DM patients.

DISCUSSION

There is strong support for a role of the type I IFN system in the pathogenesis of inflammatory myopathies (7–10). However, the cause and cell origin of the type I IFN production are unclear. Plasmacytoid DCs, known to be the major source of type I IFN, were in fact rare and scattered in muscle tissues from PM and DM patients (8). In contrast, we identified CD56+ and HLA class I+ immature muscle cells to be a major source of IFNβ.

The role of muscle fibers in the pathogenesis of inflammatory myopathies has been suggested by many studies, which have implicated immature regenerating muscle cells, the up-regulation of HLA class I antigens, and the activation of the TLR pathway (11,13–15). This in vitro study confirmed our previous study indicating a small production of IFNβ by cultured myoblasts after activation of the TLR-3 pathway and in the presence of the Th1 cytokine IFNγ. In turn, the local production of type I IFN by muscle cells may contribute to the up-regulation of HLA class I antigens on muscle cells, which may be one of the mechanisms of muscle fiber damage (14). The induction of HLA class I expression by poly(I-C) was partly dependent on autocrine IFNβ. It should be noted that the partial inhibition of poly(I-C)–induced HLA class I expression after IFNβ neutralization may be related to a partial inhibitory effect of the neutralizing antibody or to an IFNβ-independent pathway.

An important development in the field of innate immunity has been the identification of pattern-recognition receptors (PRRs), including TLRs and CLRs, which are a critical link to the innate and adaptive immune responses. DCs, which are equipped with a range of PRRs, such as CLRs, play a central role in the formation of the immune response. Activation of DCs and monocytes through PRRs results in the differentiation into mature DCs with distinct phenotypes. These distinct DCs promote specific types of T cell response. We have previously shown the involvement of the innate immune system in PM and DM through the activation of the TLR pathway (11).

In addition to the overexpression of TLR-3 and TLR-7 in PM and DM (11), the present study showed the overexpression of DC-SIGN, MMR, and ASGPR, the main CLRs expressed by DC/macrophage lineage cells in inflammatory myopathic tissues. In samples from PM and DM patients, their expression was predominantly localized in inflammatory infiltrates and associated with the presence of IL-12 and IL-23. The IL-12 and IL-23 cytokines are products of activated DCs and differentially promote the polarization of T cells into Th1 and Th17 cells, respectively (23). This is consistent with the detection of both Th1 and Th17 cells in lymphocytic infiltrates of PM and DM muscle tissues (1,2). A dominant Th1 profile in inflammatory myopa-

thies (3–6) is consistent with the TLR-3 overexpression we have previously observed (11) and contributes to the local production of IFNβ after TLR-3 activation. Such production of IFNβ after TLR-3 activation contributes to the HLA class I up-regulation. Furthermore, increases in necrotic cell numbers during chronic inflammation and activation of the TLR-3 pathway possibly by exogenous and/or endogenous activators released from damaged cells (11), combined with Th1 cytokines, amplify the induction of IFNβ and contribute to the formation of a self-sustained inflammatory loop. Such induction of type I IFN could be further increased by immune complexes composed of myositis autoantibodies (8). This is consistent with previous reports of an increased expression of myositis autoantigens in regenerating muscle cells (15).

Finally, these results strengthen the critical role of immature muscle fibers in the pathogenesis of PM and DM. When we examined CD56, a classic marker of immature myoblasts, as well as HLA class I expression, a key marker of myositis, we found that inflammatory infiltrates with CLR-expressing cells were localized around muscle cells expressing high levels of CD56 and HLA class I antigens. This suggests that immature muscle precursors may represent the target of an immune response involving both the innate and adaptive immune systems. Thus, by focusing damage on the immature muscle cells that are in charge of repair, the muscle wasting and the reason why muscle regeneration is not effective in the context of such inflammation could be explained. It appears that inflamed muscle from patients with PM and DM is characterized by the overexpression of HLA class I antigens, myositis autoantigens, TLR-3, TLR-7, and type I IFN, all of which are localized to immature muscle precursors (11,15).

In conclusion, our results indicate that immature muscle precursors are a possible source of type I IFN. These muscle cells, which are usually responsible for the regenerating process, may be the target of the immune attack. Moreover, activation of the TLR-3 pathway in combination with the Th1 cytokine IFNγ may be involved in HLA class I overexpression through the induction of IFNβ.

ACKNOWLEDGMENTS

We thank Professor P. Dechelotte and Dr. A. M. Beaufrere (Laboratory of Pathology, CHU Clermont-Ferrand) for providing access to muscle biopsy samples and for technical assistance.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Miossec had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Tournadre, Eljaafari, Miossec.

Acquisition of data. Tournadre, Lenief, Eljaafari.

Analysis and interpretation of data. Tournadre, Lenief, Eljaafari, Miossec.

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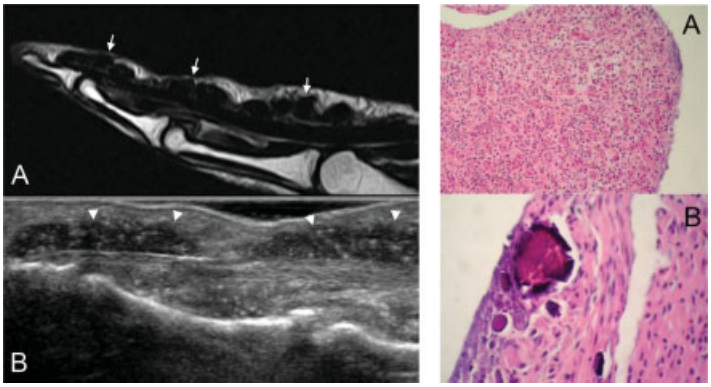
DOI 10.1002/art.33429

Clinical Images: Steroid-induced chemical synovitis



The patient, a 34-year-old woman, had a 16-year history of treatment-refractory systemic lupus erythematosus, which had evolved to lupus nephropathy with chronic renal failure necessitating peritoneal dialysis, and lupus cardiomyopathy. She had initially been treated with hydroxychloroquine and prednisone; due to lack of response, the treatment was then switched to azathioprine and later to cyclophosphamide and pulse methylprednisolone, with incomplete response. She presented with a 3-month history of right palmar pain localized to the index finger flexor, which was not responsive to nonsteroidal antiinflammatory drug and systemic steroid treatment. Local steroid treatment was administered (betamethasone dipriopionate and betamethasone disodium phosphate solution, 1 cc), but within 2 days after the injection the pain worsened and swelling developed. Magnetic resonance imaging (MRI) of the hand was performed (**A,** left) (T2-weighted sagittal view), which revealed marked distension with lobulated margins of the synovial sheath of the flexor tendons of the middle finger (**arrows**) with predominantly hypointense T2 signal, associated with mild peritendon edema. These findings were suggestive of severe tenosynovitis associated with the presence of microcrystals in the synovial sheath. On the same day an ultrasound was performed (**B,** left), which confirmed the MRI findings, showing marked distension of the synovial sheath and multiple small hyperechoic foci around the tendon (**arrowheads**). Due to persistence of the symptoms, surgical excision was performed. Biopsy results confirmed the presence of chemical tenosynovitis secondary to steroid crystal deposition, with signs of synovial membrane hyperplasia and mixed inflammatory infiltration (**A,** right) (hematoxylin and eosin stained; original magnification × 40). A higher-power view revealed the basophilic crystalloid deposit, surrounded by macrophages (**B,** right) (original magnification × 400). In contradistinction to steroid flare (a relatively common complication, which causes pain that is usually self-limited), chemical tenosynovitis is much less common, with longer-lasting and more severe pain and swelling that may require surgical treatment.

Oscar E. Contreras, MD
Alvaro J. Burdiles, MD
Maria C. Vial, MD, MSc
Pablo F. Riquelme, MD
Pontificia Universidad Catolica de Chile
Santiago, Chile